



THE AMERICAN JOURNAL  
OF PATHOLOGY





# THE AMERICAN JOURNAL OF PATHOLOGY

*Official Publication of  
The American Association of Pathologists and Bacteriologists*

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## VOLUME XII

## 1936



BOSTON  
MASSACHUSETTS  
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65444

PRINTED AT THE HARVARD UNIVERSITY PRESS  
CAMBRIDGE, MASS., U. S. A.

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# THE AMERICAN JOURNAL OF PATHOLOGY

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VOLUME XII

JANUARY, 1936

NUMBER I

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## THE PATHOLOGICAL CHANGES IN THE BONE MARROW IN AGRANULOCYTOSIS \*

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One of the problems that confronts the hematologist of today is the question of the identity of agranulocytosis as a disease entity. The specificity of the condition has by some been vigorously denied, by others emphatically asserted, and by others again regarded as irrelevant or unimportant.

Evidence on this question may be garnered from both clinical and pathological view-points. This paper concerns itself with the latter.

In the medical literature of the pathology of agranulocytosis one finds almost complete chaos. This situation would appear to arise in part from an uncritical choice of cases studied, and in part from lack of appreciation of such variations in cellular composition as normally occur from one bone to another, even in the strictly normal individual. Thus Aubertin and Lévy<sup>1</sup> make no attempt to separate agranulocytosis pure and simple from pancytopenia, aplastic anemia and allied diseases, or from those leukopenic states obviously secondary to known toxic agents. Dodd and Wilkinson<sup>2</sup> describe the marrow of an 11 year old congenital syphilitic negro who died subsequent to arsphenamine therapy. Dameshek and Ingall<sup>3</sup> report 9 cases with pathological considerations drawn from 2. One had a progressive and eventually extreme anemia, the red blood cell count falling within a month from 4,000,000 per cmm. to 850,000 per cmm. The

\* Received for publication August 17, 1935.



2nd case was a girl 4 years old with severe sepsis, bleeding from the mucous membranes, and a red blood cell count of 1,500,000 per cmm. Of the white blood cells 20 to 40 per cent were immature forms. Zadek <sup>4</sup> emphasized the presence of lymphocytes in the bone marrow and interprets the finding as indicating an active infiltration crowding out the myeloid cells. Yet, 2 of his cases were almost certainly leukemic in nature and showed progressive anemia and infiltration of the organs generally with large, immature lymphocytes, and a 3rd case showed a lymphocytosis of 11,000 per cmm. and lymphocytic nodules in the bone marrow. Such cases are instructive and serve to caution us against too great dogmatism regarding the pathological changes in leukopenic states. That they advance our knowledge of the pathology of agranulocytosis may be questioned.

On the other hand, Campbell and Murdock <sup>5</sup> describe the marrow of the tibia in their case (which incidentally was secondary to pneumonia) as fatty, without apparent recognition of the fact that the bone marrow from this region is normally fatty except in early childhood. Schultz, himself, has frequently been quoted as regarding the bone marrow in agranulocytosis as aplastic, yet Leon, <sup>6</sup> who reported on the histological changes in Schultz' cases, confines herself to the bare statement that neither adult polymorphonuclear neutrophils nor myelocytes were seen and that grossly the femur was partly red and partly fatty. Lichtenstein <sup>7</sup> in a most complete article reporting bone marrow studies in 18 of his own cases found a fairly uniform picture in the diaphysis of the femur, polymorphonuclear neutrophils absent or very rare, myelocytes usually entirely absent, myeloblasts present in small, occasionally moderate numbers, scattered lymphocytes, normal or increased numbers of megakaryocytes and plentiful red cells in all stages of development. Koch, <sup>8</sup> Kommerell, <sup>9</sup> and Baltzer <sup>10</sup> find in smaller groups of cases similar pictures, with the exception that plasma cells are also present in large numbers.

A further group of authors emphasize degenerative changes in the myeloid cells. Rotter <sup>11</sup> found many degenerated myeloblasts in the marrow of 1 of his cases. On this basis he has been quoted, without qualification, as finding degenerated myeloblasts in the marrow of agranulocytosis. This patient, however, clinically had a marked anemia and according to the author himself was questionably diagnosed agranulocytosis. Moreover, the marrow at autopsy was grossly invaded by bacteria, a fact which might well explain the observed

degeneration. Oppikofer<sup>12</sup> describes in 3 cases a few to many degenerated and degenerating myeloblasts. Photomicrographs accompanying his article show degenerated cells, the identification of which as myeloblasts is impossible from the photographs. Roberts and Kracke,<sup>13</sup> Hartwich,<sup>14</sup> Uffenorde,<sup>15</sup> and Pepper<sup>16</sup> concur in the observation of this degeneration in at least some of their cases. More recently Jaffé<sup>17</sup> has still further emphasized degeneration of the myeloid cells, especially the myelocytes in which he says the granules "swell and fuse with the cytoplasm."

The confusing influence of such varied reports is patent. Cases are described with the most diverse clinical and hematological pictures and the essential pathological bone marrow change of "agranulocytosis" has variously been described as aplasia, lymphoid infiltration and degeneration of existing cells.

It remained for Fitz-Hugh and Krumbhaar<sup>18</sup> to clarify the situation in their excellent study of agranulocytosis in 1932. They pointed out the virtually intact and unaltered state of the red cell series and, on the basis of finding a relatively cellular marrow containing, in so far as granules were concerned, only extremely early (stem) forms, they postulated for the true disease a maturation arrest of the white cells analogous to the erythroblast arrest in pernicious anemia. Their cases were for the most part typical and their pathological studies clear-cut and convincing.

These results were confirmed and amplified by the painstaking studies of Custer.<sup>19</sup> On the basis of 11 typical cases of agranulocytosis this author concluded that in the true disease the bone marrow showed a marked proliferation of myeloblasts, a failure of these cells to mature beyond that stage, a slight increase in the number of megakaryocytes and a moderate infiltration with lymphocytes and plasma cells. Custer concludes by saying that the "presence of a lesion of maturation specifically confined to the granulopoietic series, not reduplicated by diseases of known etiology, entitles idiopathic agranulocytosis, tentatively at least, to a place as a disease entity."

Some time prior to the publication of Custer's paper we had begun to collect and study all cases of agranulocytosis available to us on which we had sections of bone marrow. Only such were retained as appeared to be clinically typical and upon which we had adequate and properly prepared bone marrow sections. Typical agranulocytosis exhibits an acute onset with fever, prostration and ulcerative

stomatitis. The white blood cell count is almost invariably below 1500 per cmm. Few, if any, mature or immature granulocytes are found in the blood smear. The platelets are normal. The red blood cell count is normal or nearly so unless there be coexisting conditions producing anemia. There is no enlargement of lymph nodes not readily accounted for by adjacent sepsis, and the liver and spleen are not notably enlarged. There is little or no hemorrhagic tendency.

The group comprised 21 females and 4 males. They ranged from 24 to 85 years of age. Twenty-four died of the disease; 1 had a sternal bone marrow biopsy shortly before clinical and hematological improvement began. In all instances the leukopenia and neutropenia were extreme and unremitting. Minimum white blood cell counts varied from 120 to 1350 per cmm. with granulocytes comprising never more than 2 per cent of the total. Only 6 showed any appreciable anemia and in all this finding could adequately and logically be explained by preëxisting unrelated disease, such as chronic nephritis or advanced alcoholic cirrhosis of the liver. The lowest red blood cell count was 2,300,000 per cmm. in a patient (A-32-527) with clinical pernicious anemia which was rapidly improving under intensive liver extract therapy at the time of onset of the agranulocytosis. This case is of particular interest in view of Herndon's<sup>20</sup> report of a similar one in which, in spite of intensive liver therapy, typical agranulocytosis developed with a fatal outcome. The bone marrow, examined by Dr. William Bloom, showed no evidence of pernicious anemia but did show the characteristic changes of agranulocytosis, as described by Fitz-Hugh and Krumbhaar, and Custer. Herndon points out that such findings strongly militate against the view that liver extract may be effective in agranulocytosis and certainly evidence is afforded that the maturant factors of the red and white cell series are not identical.

None of the series showed any material reduction of platelets in the blood smear nor did any show a tendency to bleed from the mucous membranes or skin. In none could the diagnosis of aleukemic leukemia be properly entertained.

To bring out certain important similarities and differences in this series of fatal cases we have divided it into three groups on the basis of duration of symptoms before death.

The first group includes 7 cases where death occurred within 4 days of clinical onset. Typical of these is L. M. (A-32-487). This

55 year old woman, a known diabetic of some years standing, was seized rather suddenly 36 hours before entry with severe sore throat, marked dysphagia and vomiting. On admission she was found to be dehydrated and in mild acidosis, the latter feature being easily controlled by insulin and glucose. The faucial ring was injected and swollen and over the enlarged tonsils was a grayish slough. There was brawny infiltration of both sides of the neck. A few crackling râles were heard at the bases of the lungs. The red blood cell count was 5,100,000 per cmm. and the white blood cell count was 250 per cmm. In the blood smear no white blood cells other than normal lymphocytes could be found. The platelets appeared normal both in numbers and in form. The temperature was 103° F. The patient failed rapidly and died on the second day in the hospital. The bone marrow was typical of the rapidly fatal cases (Fig. 1). Red cell formation appeared to be normal. Megakaryocytes were slightly increased. No mature polymorphonuclear neutrophils or myelocytes were seen. The granulocytic elements were represented virtually entirely by stem cells (myeloblasts in Custer's terminology). Occasional scattered foci of lymphocytes occurred. Plasma cells were rare. Little phagocytosis was seen. Such cells as were present were not degenerated and mitoses were frequent among the stem cells, to which, however, further maturation appeared to have been denied.

In his rapidly fatal cases Custer found the predominating and indeed virtually the only granular cell to be the myeloblast. As a typical differential bone marrow count on such a case Custer found: myeloblasts 37.3; promyelocytes and myelocytes 2.8; red cell series 42.6; megakaryocytes 2.6; reticuloendothelial cells 9.1; lymphocytes 4.2; and plasma cells 1.3. His terminology and ours differ somewhat, but after personal communication it is obvious that we are in essential agreement. His myeloblast is our stem cell, his erythroblast our normoblast, and his normoblast our nucleated red cell. In comparison with the differential bone marrow count on a fulminating case we find on a similar case: stem cells 49; myeloblasts 7.2; lymphocytes 6; nucleated red cells, normoblasts and erythroblasts 29.4; reticular cells 1.6; and megakaryocytes 6.6. Thus, it is apparent that in those cases where death took place within 4 days of the clinical onset the essential lesion consists in an increase of the very early granular cells (stem cells) and a lack of further maturation.

For this latter phenomenon we propose the term granulocytic anaknesis.\*

The second or intermediary group including 11 patients dying from 5 to 10 days after clinical onset gives a somewhat less uniform picture in the bone marrow. Two of them, dying 7 and 10 days respectively after onset, had essentially the same picture as was seen in the earlier group.

Of the remaining 9, which may be regarded as illustrative of the intermediary group, patient F. G. (A-32-466) is typical. She was a 59 year old female who entered the hospital with a 4 day history of fever, chills, sore throat and cough. Four months previously she had been treated in the hospital for hypertension and marked chronic nephritis. Four days before the second entry she was taken ill suddenly with cough, chills, vomiting and sore throat. On entry the tonsils and pharynx were greatly injected. In the left lung were signs of bronchopneumonia. The temperature was 100° F., pulse 95, respirations 25. The urine showed a large trace of albumin and the blood non-protein nitrogen was 70 mg. per cent. The red blood cell count was 2,700,000 per cmm. with the hemoglobin 53 per cent. The white blood cell count was 900 to 1000 per cmm. on repeated determinations during her stay in the hospital. No white blood cells other than mature lymphocytes were ever seen in the blood smear. The clinical course was rapidly downhill. On the third day the pharynx showed extensive necrotic ulceration and the temperature had risen to 104° F. The throat culture showed no diphtheria bacilli or hemolytic streptococci. She died on the 8th day after the onset of disease.

Autopsy revealed a variety of pathological features: chronic glomerular nephritis, cardiac hypertrophy, old adhesive pericarditis and pleuritis, bronchopneumonia, pulmonary edema and congestion, and necrotic pharyngitis. The spleen weighed 350 gm. and showed microscopically an increase in fibrous tissue. The vertebral bone marrow, the only specimen obtained, was grossly dark red. Microscopically the cellularity was about normal, the red cell series present in normal proportions; megakaryocytes were very numerous. The myeloid series was represented by scattered stem cells and a very rare myelocyte. Polymorphonuclear neutrophils were entirely

\* This word was suggested by Dr. John H. Finley, Jr., of Harvard University and we should like to acknowledge our indebtedness to him.

absent. Plasma cells and lymphocytes were numerous, the latter usually in small clumps.

The bone marrow of the remaining 8 cases of the intermediary group could be described in the same words as this typical case — with one exception. The number of lymphocytes and plasma cells varied widely. By and large, however, in comparison with the early cases the proportion of stem cells had greatly decreased while that of the plasma cells and lymphocytes had correspondingly increased. Megakaryocytes and red cells were as before, essentially normal. Whenever sections from the femur were available, they showed a picture identical to that in the vertebra and sternum. The fruitless proliferation of stem cells had spread to the normally acellular regions and appeared to have burned itself out in a vain effort to supply to the peripheral blood normal cells of the granular series.

The third, or late, group included 6 cases where death took place after an illness of more than 10 days. A. A. (A-33-661) was typical. Six months before entry she had had a sore throat for a week, not requiring medical attention. The present illness began 6 weeks before entry with a mild sore throat which became much worse 3 weeks later. During the 2 weeks prior to admission she had had chills, high fever, headache and swollen lymph nodes in the neck, together with extreme dysphagia. On examination the tonsils were large, swollen and covered with yellow exudate. Bean-sized and tender lymph nodes were palpable in the neck. The urine contained a very slight trace of albumin. The white blood cells numbered 1000 per cmm. on entry and fell gradually to 500 per cmm. At no time were any polymorphonuclear neutrophils seen, or any early granulocytes, such as might suggest the presence of leukemia. Platelets in the smear were always very numerous. Her course was steadily downhill. The temperature was rather constantly maintained at 102° F. Pent-nucleotide (N. N. R.) was given intramuscularly in 50 cc. daily doses. On the 8th day in the hospital she died, some 3 weeks after the onset of the acute symptoms.

Autopsy showed many ulcers of tonsils, pharynx and the entire gastrointestinal tract, early bronchopneumonia, congestion and edema of lungs, liver and brain. Grossly the bone marrow from the vertebra and sternum was dark red, that from midfemur and humerus pale yellow. The vertebral sections showed an occasional stem cell, in all others they were very rare (Fig. 2). Mature granulocytes

were absent, megakaryocytes numerous. The red cell series appeared undisturbed. Most striking in all marrows were the myriads of plasma cells. In addition numerous large phagocytic cells containing red cells and nuclei were seen. Custer finds in his "marked cases" of agranulocytosis rather more myeloblasts (stem cells), more promyelocytes and myelocytes, less erythroblasts (normoblasts), and far more lymphocytes than in his "early cases." But it should be pointed out that by "marked cases" he refers to those suffering from the disease for many months. It is debatable whether these should be classed with the more acute forms. In our "late cases" — that is, in individuals dying over 10 days after the onset — we have uniformly found that the stem cells have largely given way to plasma cells, which cells now formed the predominant white cell. The red cell series still remains essentially unaltered. A typical differential count is that on JP-83a, dying 3 weeks after clinical onset: stem cells 5.2; myeloblasts 1.8; lymphocytes 19.4; plasma cells 37.4; nucleated red cells, normoblasts and erythroblasts 23.6; reticular cells 4.6; megakaryocytes 5; unclassified 3.

The remaining 5 cases of this group showed essentially identical pictures in their important features; marked myeloid hypoplasia, many plasma and lymphoid cells, essentially normal red cells, and many megakaryocytes. Only the phagocytosis was an inconstant feature, as it was marked in half the cases of this group and in a quarter of the intermediary group.

Our 1 remaining case, H. C. (S-33-3544), may best be classified as in the early recovery phase at the time of sternal biopsy. During the previous year she had had two attacks of severe sore throat without actual ulcerations. On these occasions the white blood cell count was about 2000 per cmm. with 0 to 5 per cent polymorphonuclear neutrophils. Both times she received pentnucleotide and recovered. At no time was there any anemia. The present complaint was severe sore throat for 2 days. Her throat on entry was markedly injected and she was acutely ill with a high fever. Otherwise the physical examination was normal. The white blood cell count was 1500 per cmm. with no polymorphonuclear neutrophils. That day a biopsy of the sternal marrow was taken. The next day the white blood cell count was 4450 per cmm. with 44 per cent granulocytes, mostly young polymorphonuclear neutrophils. Concomitantly she improved clinically and in 1 week she was entirely well with a white

blood cell count of 9300 per cmm. with 70 per cent polymorphonuclear neutrophils. It is interesting, in view of recent evidence that pyrimidon may be a cause of agranulocytosis, that this patient took that drug regularly before and after each attack. In spite of this regular use of the drug the white blood cell count taken periodically has been entirely normal in the past 16 months.

Microscopic study of the sternal marrow showed a picture of rapid regeneration (Fig. 3). The tissue was crowded with all stages of myeloid cells from early myelocytes to young polymorphonuclear neutrophils. Only an occasional lymphocyte and plasma cell were to be found. As might be expected, the red cell series and megakaryocytes appeared normal. The intense activity gave the impression that some stimulus had recently been given to or some block removed from myeloid development. Custer has pointed out that neither the neutropenia of overwhelming sepsis nor that of arsphenamine poisoning is accompanied by a bone marrow change similar to that described in agranulocytosis. In sepsis he found 26.6 per cent segmented polymorphonuclear neutrophils and a total of 61.8 per cent cells of the myelocyte stage or older. Similarly, we found in a case of overwhelming sepsis with a white blood cell count of 1400 per cmm. (A-35-328) 9.2 per cent segmented forms and 64.2 per cent granular cells of the myelocyte stage or older. The bone marrow picture of overwhelming sepsis is not that of agranulocytosis.

From an analysis of the 25 typical cases of agranulocytosis it would appear, as Fitz-Hugh and Krumbhaar first suggested, that in the rapidly fatal cases the bone marrow shows stem cell hyperplasia and myeloid anaknesis without notable changes in the red cell series and that as the survival of the patient becomes longer the stem cells gradually and somewhat irregularly give way to plasma cells and lymphocytes. It may be hypothesized that early in the disease there is a compensatory increase of the number of normally occurring stem cells (myeloblasts) in a vain effort to overcome the maturation arrest and that in the latter stages these stem cells disappear and a coincident increase of lymphocytes and plasma cells occurs.

#### SUMMARY AND CONCLUSIONS

1. The uniformity of the pathological changes in the bone marrow suggests that agranulocytosis is probably a disease entity.



2. Rapidly fatal cases show lack of maturation in the granular series and hyperplasia of the stem cells.

3. Cases where death took place after a longer period usually show hypoplasia of myeloid tissue with the coincident appearance of many plasma cells and lymphocytes.

4. In no case were there obvious changes in the red blood cell series, or any degenerative changes in the white blood cell series.

5. The recovery stage is characterized by rapid development of the stem cells into myelocytes, metamyelocytes and polymorphonuclear neutrophils — a sequence of events which would appear to substantiate Fitz-Hugh and Krumbhaar's and Custer's contention of a maturation arrest.

6. For this maturation arrest we suggest the term granulocytic anakmesis.

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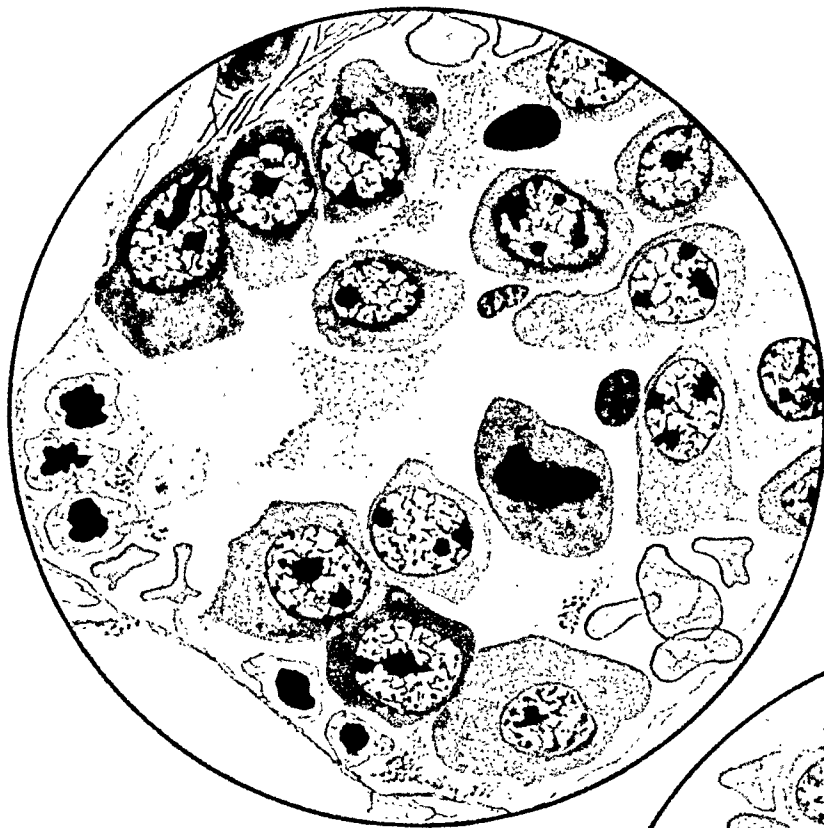
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## DESCRIPTION OF PLATE

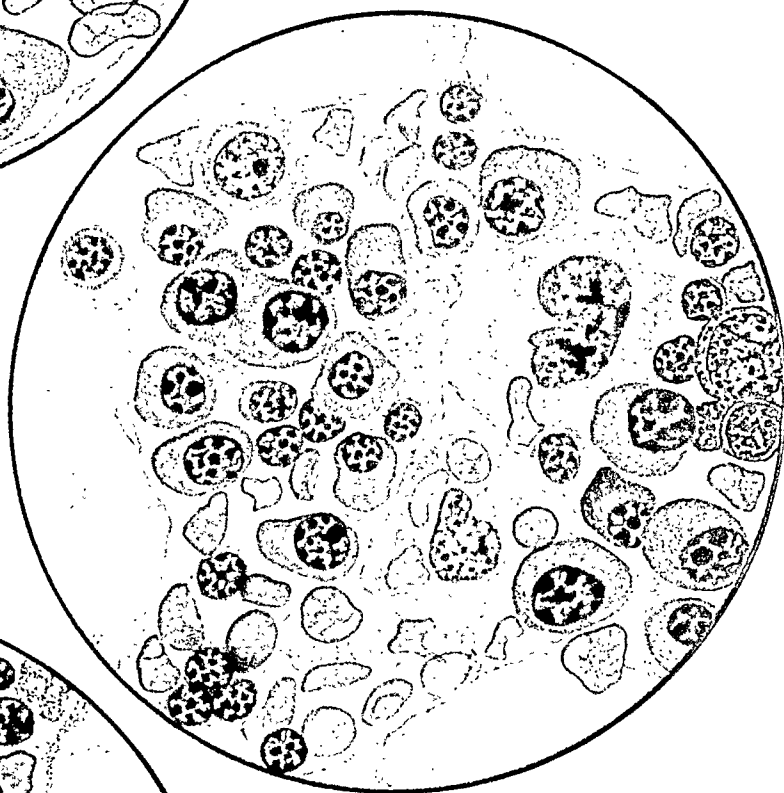
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### PLATE I

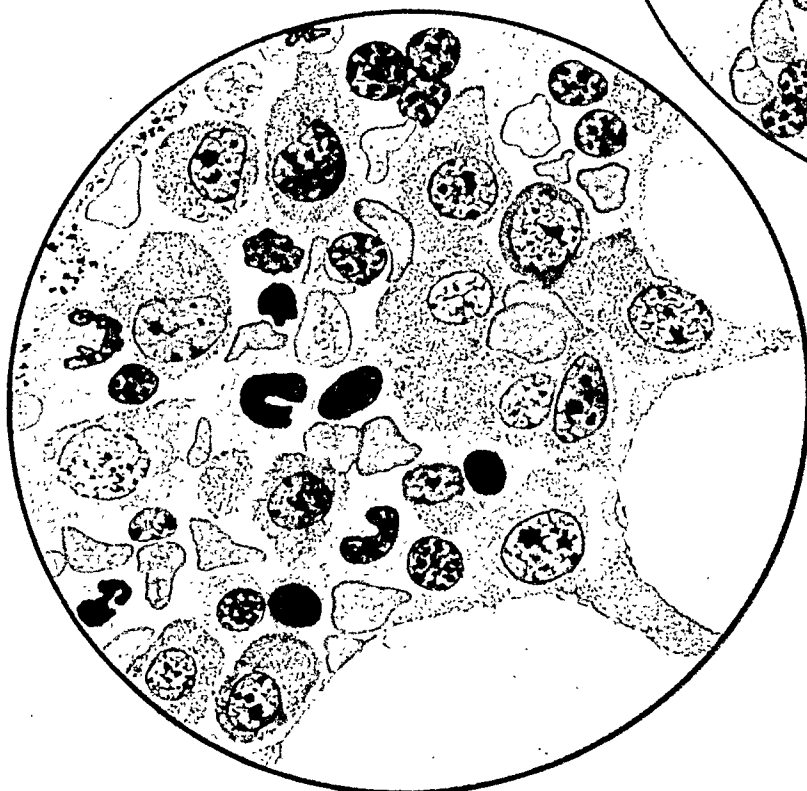
- FIG. 1. Bone marrow from a rapidly fatal case of agranulocytosis. Virtually all of the white cells are stem cells, some showing active mitosis. (A-32-13.)  $\times 1000$ .
- FIG. 2. Bone marrow from a case of agranulocytosis where death took place 3 weeks after clinical onset. Stem cells are virtually entirely replaced by plasma cells and lymphocytes. One phagocytic cell engulfing red cell. (J P-83a.)  $\times 1000$ .
- FIG. 3. Bone marrow from a case of agranulocytosis just prior to improvement in the peripheral blood picture. Rapid development of myelocytes with the presence of an occasional young polymorphonuclear neutrophil. (J P-74.)  $\times 1000$ .



1



2



3

Darling, Parker, Jackson

Bone Marrow in Agranulocytosis



# A SPECTROGRAPHIC STUDY OF LEPROUS LESIONS \*

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These determinations were made with the idea that significant modifications might occur in Na, Ca, Mg, P, Fe and, perhaps, other elements in the lesions as compared with unaltered tissue of the same sort. It was thought that if a marked chemical change paralleled the development of distinctive lesions, something might be done to re-establish normal ratios between the elements that would be of therapeutic value. In this paper we wish to report a beginning in this direction made possible by the fact that histopathological studies on leprosy are being carried on in the same laboratory in which Dr. Gordon H. Scott and his associates are using the technique of histospectrography.<sup>1-4</sup> We are very grateful to Dr. Scott for his help and advice. We have made a spectrographic examination of leper lesions from 6 cases, the examination involving comparison with five "normal" skins taken to represent roughly normal conditions, and with three other such skins on which chemical analyses were run. All eight of the controls afford opportunity for semiquantitative estimates (of the "greater than" or "less than" variety as described by Scott and Williams<sup>2</sup>) of P/Ca, Na/Ca, Mg/Ca and Fe/Ca ratios in leper lesions as compared with normal skin; and we have worked out numerical values for the P/Ca and Na/Ca ratios based upon the spectrographic findings on three chemically analyzed normal skins.

## MATERIAL

The leper tissues were collected at biopsy from five lepers at the United States Marine Hospital, Carville, Louisiana, with the permission and cooperation of Dr. O. E. Denney and through the kindness of Major S. Simmons, at autopsy by Dr. E. DeCoursey of the Board of Health Laboratory, Ancon, Canal Zone. The former are referred to as L 11 to L 15. The lesion in each was divided into five pieces which were qualitatively alike as far as could be determined

\* Aided by grants from the Josiah Macy, Jr. Foundation and the Leonard Wood Memorial.

Received for publication September 17, 1935.

macroscopically. These were immediately fixed (1) in 10 per cent formalin in absolute alcohol, (2) 10 per cent formalin, (3) Regaud's fluid (3 per cent potassium bichromate 4 parts, and formalin 1 part), (4) formalin-Zenker's for histological examination and (5) frozen with dry ice and kept frozen until studied spectrographically in St. Louis. The latter, designated L 3 and L S 3, were fixed in the formalin-alcohol mixture and mailed to St. Louis therein. On arrival equivalent parts were used for histological and spectroscopic examination. It is necessary to specify the source and the histopathology of the particular lesions studied spectroscopically because others of different nature may have a different mineral content.

L 11. Patient P. A. 1049, Mexican, male, 42 years of age, mixed leprosy with cutaneous type predominating, 4 years duration with no treatment. *Biopsy*: removal of the ear lobules.

*Epidermis*: Slight hyperkeratosis; 35-75  $\mu$  thick, average 45  $\mu$ . Considerable pigment. No leukocytic invasion. No epithelial pegs.

*Papillary Layer*: Almost obliterated owing to absence of pegs and flattening of dermal papillae. The remains of the layer constitute the subepithelial marginal band of Unna.<sup>5</sup> The thickness of this band varies from 36-40  $\mu$ . It is made up of collagenic fibers with a few fibroblasts between them. The elastic fibers, normally present in the papillary layer, are much reduced in number and no traces remain of the sense organs of the dermal papillae in any of these leprosy specimens. The band has a blood supply greatly reduced from that of the original papillary layer, but it is separated from the underlying reticular layer by a zone of moderately distended vessels. The relative immunity of this subepithelial marginal band to penetration by the leproma cells, emphasized by Unna, holds for this and other specimens in our series.

*Reticular Layer*: Much thickened by a leprosy nodule, which is only partly divided into lobules by septa of connective tissue (seldom more than 75  $\mu$  wide), blood vessels (about normal in size), many sebaceous glands and hair follicles. The latter are slightly atrophic. The nodules are made up chiefly of histiocytes, foam cells, lymphocytes, fibroblasts, polymorphonuclear leukocytes, tissue mast cells and tissue eosinophiles, cited in order of frequency. The term "plasma cell" is used in the sense of von Marschalkó,<sup>6</sup> which is different from that of Unna, who employed it as synonymous with "protoplasmic cells," which clearly include histiocytes (macrophages, monocytes, epithelioid cells, and so on) as well. No giant cells like those illustrated by Wade's<sup>7</sup> Figure 12 were seen in this or in specimens from any of the other cases. Bacilli are numerous in the histiocytes as typical "cigar packs," or intracellular globi. Globi of the same sort are found in a few fibroblasts and occasionally in vascular endothelial cells. Bacilli, not clumped as globi, are occasional in the outer cells of sebaceous glands, duct cells of sweat glands and in the tissue fluid. Much larger, roughly globular masses of bacilli, which are not intracellular but intercellular and have been called "giant globi" by Unna, are rare. Denney has commented upon them in

a recent paper.<sup>8</sup> We hope to present in another communication our views as to their nature, relation to the small intracellular globi, and the whole very important question of lymphatic involvement. Here we are concerned only with their occurrence and size in an attempt to grade the tissues. In L 11 they were not numerous and the largest had a maximum diameter of  $15\mu$ .

Only a small amount of *subcutis* is included in the sections. Contrasted with other specimens, nodule formation is the least advanced, giant globi least frequent and the normal structure of the skin least disturbed. Conversely, L 11 exhibits more fatty areolar tissue, well formed sebaceous glands, and in the nodules a higher percentage of tissue mast cells and plasma cells than any of the others.

L 12. Patient W. M. 619, white, male, 30 years of age, mixed leprosy with cutaneous type predominating, 10 years duration under routine chaulmoogra oil treatment. *Biopsy*: removal of nodule on forearm.

*Epidermis*: Atrophic, tendency to hyperkeratosis;  $30-135\mu$  thick, average  $95\mu$ . Little or no pigment. Slight leukocytic invasion. Epithelial cells swollen, increase in size approximately 33 per cent. No epithelial pegs.

*Papillary Layer*: Ironed out owing to lack of pegs and dermal papillae. The subepithelial marginal band is  $10-150\mu$  wide, much less dense than in L 11 and limited internally by a zone of dilated vessels.

*Reticular Layer*: The leprous nodule is of fairly uniform consistence, since it is not broken up by sebaceous glands, hair follicles, fatty areolar tissue or large bands of connective tissue; for all these are absent. The sweat glands are atrophic but the blood vessels are not noticeably changed. The nodule is more cellular and less fibrous than in L 11. The connective tissue increases gradually as the subcutis is approached and becomes disposed in strata and whorls. In the nodule, intercellular spaces are marked, particularly near the external margin, suggestive of much tissue fluid, more indeed than in any of the other specimens. The most abundant cells in the nodules are histiocytes. There are many foam cells, a few lymphocytes and occasional polymorphonuclears. Plasma cells are rare and no tissue mast cells or tissue eosinophiles are seen. Bacilli are abundant as small intracellular globi in histiocytes and free in the tissue. Giant globi are fairly numerous. The walls of the lymphatics, which contain them, show more marked hyperplasia than in any of the other specimens except L 3, so that when cut at an angle they simulate giant cells. No true polykaryocytes are seen. The *subcutis* is not included in section.

L 13. Patient F. H. 971, Mexican, male, 36 years of age, with marked cutaneous leprosy of 5 years duration. Has had no treatment. *Biopsy*: removal of nodule on forearm.

*Epidermis*:  $20-80\mu$  thick, average  $35\mu$ . In one area, about 1 mm. wide, it is distinctly atrophic being approximately  $20\mu$  thick. The cells are largest and vacuolated where the epithelium is thickest. Much pigment. No leukocytic invasion. No epithelial pegs.



*Papillary Layer:* Almost flattened out by absence of epithelial pegs and great reduction in size of dermal papillae, which only penetrate epidermis to depth of 20 to 50  $\mu$  and are not numerous. The subepithelial marginal band is about half the thickness of the overlying epidermis. It is mostly collagenic, but in its outer lamina rather more elastic fibers are demonstrable by resorcin fuchsin than in the other cases. A few pigment cells are present in the band.

*Reticular Layer:* Thickened, by growth of leprous nodule which is not much separated into lobules by connective tissue or epithelial derivatives. No sebaceous glands or hair follicles. On the average there is only one duct of sweat gland per section, the lumen of which is almost closed. In the nodule, histiocytes are most numerous, plasma and foam cells are rare, the blood vessels are open with normal walls. No tissue mast cells or eosinophiles were seen but long search was not made. Bacilli are quite granular, but very numerous especially as small globi intracellular in histiocytes. There are many giant intercellular globi. The largest was 50  $\mu$  in diameter (measured in 10 per cent formalin-fixed material). Only one instance of proliferation of lymphatic endothelium leading to pseudo-giant cell formation was noted.

*Subcutis:* Only partly included in section. No fatty areolar tissue anywhere in specimen.

L 14. Patient F. V. 514, white, male, 35 years of age, mixed type of leprosy with cutaneous lesions predominating, 19 years duration, routine treatment with chaulmoogra oil. *Biopsy:* removal of nodule from nape of neck.

*Epidermis:* Atrophic and smooth; 25-40  $\mu$  thick, average 30  $\mu$ . Small amount of pigment. No leukocytic invasion or pegs.

*Papillary Layer:* This is practically absent, because the pegs and dermal papillae are lacking. The subepithelial marginal band is narrower and less pronounced than in any other specimens of the series. It varies in width from 10-25  $\mu$ . In some cases the cells of the nodule come into direct contact with the inner surface of epithelium. But in that portion fixed in alcohol-formalin the band is wider and more like tissue from the other cases.

*Reticular Layer:* Enlarged by nodule formation. Not broken up into lobules by hair follicles, sebaceous glands or sweat glands; for these are absent, except in the alcohol-formalin-fixed piece in which there are two small sebaceous cysts. It consists of histiocytes, foam cells in moderate numbers, a few lymphocytes, plasma cells, and occasional polymorphonuclears together with fibroblasts and connective tissue fibers disposed in thin bands. There are no tissue mast cells or eosinophiles. Bacilli are very numerous, free and in small globi. Giant globi are more frequent in the Regaud-fixed fragment than in specimens from any of the other cases, but in other pieces from this case they are not unusually abundant. No *subcutis* is available. No fatty areolar tissue or giant cells are seen.

L 15. Patient J. P. 1050, Mexican, male, 30 years of age, mixed type of leprosy with cutaneous lesions markedly predominant, 8 years duration with no treatment. *Biopsy:* removal of nodules from face.

*Epidermis:* Atrophic, 30–55  $\mu$  thick, average 40  $\mu$ . It contains considerable pigment. Leukocytic invasion is very rare and there are no pegs.

*Papillary Layer:* Reduced by absence of pegs and dermal papillae. The subepithelial marginal band is 30–100  $\mu$  thick and chiefly collagenic. The most dilated venules in the series are just internal to the band but the number of vessels is not increased.

*Reticular Layer:* Greatly increased in thickness by nodule formation. The nodule is of fairly uniform consistence, being very little broken up by sebaceous glands and hair follicles which are distinctly rare. Connective tissue bands are but feebly developed and there is no fatty areolar tissue except in the fragment fixed in formalin-Zenker in which the included lesion is less severe. The nodule in all specimens of L 15 is made up of histiocytes, with plasma cells more numerous than in any of the other cases except L 11, lymphocytes, foam cells and a few tissue mast cells; but only one multinucleated giant cell much vacuolated and about 35  $\mu$  in maximum diameter was seen. Bacilli are numerous, free, in small globi and in giant globi. The latter, though not particularly frequent, are the largest seen in the series of leprosy specimens. One was oval in shape in section with maximum diameter of 125  $\mu$  and minimum of 108  $\mu$ . In general, tissue from this case is rather like that of L 11.

### L 3. Autopsy No. 10563, Board of Health Laboratory, Ancon, Canal Zone.

*Epidermis:* 20–80  $\mu$  thick, average about 50  $\mu$ . Heavy pigmentation. No leukocytic invasion. Inner surface is very uneven owing to extension of numerous short (20–120  $\mu$ , average 70  $\mu$ ), pointed epithelial pegs — a feature more marked in this than in any of the other specimens.

*Papillary Layer:* Atrophied as compared with normal, but not ironed out, like many of the others, because dermal papillae alternate with the pegs. It contains more than the usual number of pigment-holding cells. The subepithelial marginal band is indistinct. Increase in collagenic fibers is only moderate and decrease in elastic fibers is limited to approximately the inner half of the reticularis. Moreover, the thickness of the band is variable (30–100  $\mu$ , average 70  $\mu$ ) depending on the proximity of the nodules to the epithelium.

*Reticular Layer:* Contains layer of relatively small flattened nodules, each having a length (parallel to epidermis) of about 150–800  $\mu$  and maximum thicknesses of 40–300  $\mu$ . Between them stretch bands of connective tissue, blood vessels, a few nerve fibers and the ducts of sweat glands. The nodules are backed internally by a feltwork of thick strands of connective tissue containing many elastic fibers. In the feltwork are nodules of the same and larger size which extend toward the subcutis as far as the section goes, namely 3–4 mm., and are accompanied by fatty areolar tissue in amount only slightly less than normal. The composition of the nodules differs. Those of the outermost layer are made up for the most part of foam cells and histiocytes, the former being most numerous. Lymphocytes and plasma cells are scarce. Only two tissue mast cells and no eosinophiles were seen. In the deeper nodules there are with the foam cells, a fair number of lymphocytes and plasma cells, some fat, many giant globi attaining a maximum diameter of 50  $\mu$ , and numerous giant cells whose maximum diameter is about 70  $\mu$  and whose largest number of nuclei in a 6  $\mu$  section is 10 per cell. In some cases, the giant cells are merely sections through hyperplastic

walls of lymphatics in which cell boundaries are lost. Bacilli in both superficial and deep nodules are granular and fragmented.

L S 3 is from the same autopsy as L 3, selected because on gross inspection it showed so few signs of leprous change that the results of spectrographic examination would be significant as compared with L 3.

*Epidermis:* Similar.

*Papillary Layer:* Similar but slightly more elastic tissue remaining and tissue mast cells distinctly more numerous.

*Reticular Layer:* Outer layer of small nodules similar. Deeper nodules less than half as large. The cytology of the nodules is similar, except that there are fewer giant globi and giant cells than in L 3.

The normal tissues consisted of pieces of skin removed from the upper left chest of five white cadavers, age about 60 years or more. These are known as N 1 to N 5 inclusive. In addition, skin from three individuals was analyzed chemically for P, Na and Ca by Miss C. C. Buhrmester, as well as studied spectrographically. The specimens were from the abdomen of a male and female cadaver of about the same age as N 1 to N 5 and from the chest of a male, aged 53, who died of empyema (our Department of Pathology Autopsy No. 6184), obtained through the courtesy of Dr. H. L. McCordock. These are called M, F and A, respectively. We are grateful to Dr. R. J. Terry for permission to collect skin from the cadavers.

## SPECTROGRAPHIC ANALYSES

### (A) *Handling of Material*

The method of obtaining the spectra of the materials has been fully described elsewhere.<sup>1, 2, 3</sup> It consists essentially of burning 2-4 cmm. (per spectrum of 15" exposure) of tissue in a high frequency spark and photographing the emission spectrum with a small quartz spectrograph. The material to be burned was first cleansed of subcutaneous fat by scraping with a knife, then cut in strips about 2 mm. square, and up to a centimeter or two in length, in the case of normal skin. With the leprous lesions, however, the strips were necessarily somewhat shorter. They were held in small pyrex glass tubes for introduction into the spark. The portion of each leper lesion selected was always immediately adjacent to those examined histologically. With each of the normal skin cases M, F and A, the

strips for the spectrographic record were taken at three different places from the rather large area of skin (25 to 100 sq. cm.) used for the chemical analysis. The whole area of skin was cleansed of subcutaneous fat before the strips were cut.

The spectra, in general, show spectral lines characteristic of Ca, K, Na, Cu, Mg, Fe, P, Si, C and occasionally Ag. These lines are visible on the accompanying plates, which exhibit representative portions of the sets of spectra obtained for the materials employed. Beside the spectra are listed the designations employed in the description of material. Only the lines actually measured up are indicated — for the others, see previous papers.<sup>1,2,3</sup> Also very evident are the continuous bands due to the passage of the spark through the air.

The density ( $= \log_{10}$  opacity) of one line each for P, Na, Ca, Mg and Fe was measured with an electrical densitometer<sup>4</sup> in each spectrum examined. The results were then averaged for each kind of tissue. For instance, the four spectra available for leper Case 11 yielded four values for the P line density, and the average of these gave the P line density characteristic of the case. The results of such treatment of all the densitometer readings are given in Table I, which indicates also the number of spectra measured for each material. All spectra were taken with the same exposure time (15 seconds) and on the same type of plate (Eastman 50).

### *(B) General Quantitative Estimates*

The working hypothesis is: If in the spectra of two similar tissues A and B, the Mg lines are of about the same density, while the P lines of A are much more dense than those of B, it can be concluded that the P/Mg ratio is greater for A than for B. Any other two elements can be taken, besides Mg and P. It is not sufficient to fix attention on the lines of only one element, since errors would ensue from the considerable variations in the rate of consumption of material by the spark. It is assumed that the spectra to be compared are taken on the same type of plate and with the same exposure time, and given as nearly as possible the same development. The spectra here considered do not very well satisfy the development requirement, since they were taken over an interval of some months without particular reference to the scheme of presentation now employed. However,

the relations to be pointed out are sufficiently pronounced not to be invalidated by this criticism.

The hypothesis requires that with a given general type of tissue (for example, skin, which burns brightly, as distinct from fat, which melts and sputters in the spark) the line intensity increases with increasing amounts of the element in the tissue. With the low metallic element concentrations encountered in nearly all tissues, and with the large amount of organic material present which tends to "ballast" spark phenomena against alteration due to changes in metallic content, self-reversal and quenching of the spectral lines of the elements of interest should be slight; consequently, the requirement should be satisfied.

The outstanding difference between leper and normal tissue seems, for the cases at hand, to concern calcium and phosphorus. From Table I the average Ca line density for leper Cases 11-15 is 0.65, while for the eight normals it is 0.76; but the P relations are in the other direction — leper 0.67, normal 0.44. Hence we conclude the P/Ca ratio is larger for leper lesions than for normal skin. This can be seen in comparing Figures 1 and 2 for relative P and Ca line strengths. Almost the same numerical relations exist for the Ca and Fe line densities, and hence the same conclusion follows for the Fe/Ca ratios, see again Figures 1 and 2. While inspection of the Ca and Mg densities indicates a tendency for the Mg/Ca ratio to be greater for the leper material, the differences are perhaps not large enough to establish anything in view of the large variations observed from sample to sample of the same type of material, and in view of possible errors arising from the fact that the spectra concerned were on various plates. Likewise, the Na/Ca ratios show only a tendency to be greater in the leper material.

Leper Case 3 is of special interest. Here we have spectra of two lesions of the same type but of different severity from the same patient on the same plate. Further, both samples were taken at autopsy. Thus the comparison is free from certain of the qualifications which apply to those already mentioned. In the first place it is very evident, not only from Table I, but also from Figure 1, that the P/Ca ratio is greater for the heavier lesion (L 3) than for the lighter one (L S 3). The Mg/Ca ratio shows again the tendency in that direction, as does the Na/Ca ratio. But the Fe/Ca ratio seems to be the same in both.

This last point, perhaps, accounts for the high Fe/Ca ratio in leper Cases 11-15 as compared with the normals; the former samples were taken at biopsy; the latter generally months after death, while both samples for Case 3 were taken at autopsy at the same time. Since the spectrum of whole blood (Scott and Williams,<sup>2</sup> Figure 4, Spectrum 1) has been found to be extremely rich in Fe from the hemoglobin, it is probable that in spite of the low vascularity of leper lesions enough blood was retained after biopsy to account for the

TABLE I

*Mean Line Densities for Spectra of Leper and Normal Tissues*

Case	Number spectra	Line densities				
		Ca	Mg	P	Fe	Na
Leper L 11 .....	4	0.73	0.51	0.62	0.76	0.3
L 12 .....	4	0.54	0.51	0.66	0.51	0.82
L 13 .....	4	0.70	0.55	0.66	0.82	0.91
L 14 .....	3	0.58	0.51	0.66	0.66	0.82
L 15 .....	3	0.69	0.64	0.77	0.66	0.89
Average .....		0.65	0.54	0.67	0.68	0.85
Normal N <sub>1</sub> .....	4	0.73	0.53	0.38	0.34	0.78
N <sub>2</sub> .....	4	0.79	0.46	0.36	0.41	0.74
N <sub>3</sub> .....	4	0.63	0.44	0.40	0.32	0.84
N <sub>4</sub> .....	4	0.67	0.34	0.27	0.31	0.70
N <sub>5</sub> .....	4	0.87	0.48	0.41	0.38	0.72
M .....	12	0.86	0.44	0.72	0.71	1.04
F .....	12	0.93	0.56	0.67	0.73	1.07
A .....	10	0.68	0.37	0.36	0.38	0.88
Average .....		0.76	0.45	0.44	0.45	0.85
Leper L 3 .....	10	0.96	0.83	0.87	0.76	0.92
L S 3 .....	10	0.94	0.73	0.58	0.73	0.75

strong Fe lines in the leper spectra, particularly as the samples were immediately frozen in solid CO<sub>2</sub> rather than preserved in a liquid fixative. It should be remembered, also, that the normals were taken from the chest or abdomen in cadavers which had been hanging vertically for some time, and signs of extensive blood drainage into the lower portions of the bodies were evident.

Finally it is instructive to apply the above method of interpretation, for the P/Ca and Na/Ca ratios, to the normal skin cases M, F and A, for which these ratios were determined chemically (Table II, columns 5 and 7). Referring again to Table I, Ca (M) < Ca (F),

while  $P(M) > P(F)$ ; hence,  $P/Ca$  for  $M$  is greater than for  $F$ , which checks the chemical results. The comparison of  $A$  with either  $M$  or  $F$  is not quite of the same sort, and must be handled differently. We may note that the difference of the  $P$  and  $Ca$  line densities for  $A$ , ( $0.36 - 0.68 = -0.32$ ) is less algebraically than the corresponding difference for either  $M$  or  $F$  ( $-0.14$  and  $-0.26$ ). Since the smaller the amount of  $P$  present in proportion to the  $Ca$ , the smaller should be this difference, we again check the chemical results. A similar treatment holds for the  $Na/Ca$  relation. The differences in  $Na$  and  $Ca$  line densities for  $M$ ,  $F$  and  $A$  are respectively  $0.18$ ,  $0.14$  and  $0.20$  which are in the same sequence as the chemical findings  $14.1$ ,  $10.5$  and  $18.1$  for the  $Na/Ca$  ratios.

### (C) *Numerical Estimates*

In the foregoing we used the difference in the  $P$  and  $Ca$  line densities for a particular kind of tissue as an index for the  $P/Ca$  ratio in that tissue, and similarly for  $Na/Ca$ . The qualitative validity of this choice of index was shown by the agreement obtained for the normals  $M$ ,  $F$  and  $A$  with the chemical findings. We shall now correlate the indices for  $M$ ,  $F$  and  $A$  numerically with the chemical results, and by graphical methods find the  $P/Ca$  and  $Na/Ca$  ratios for the other materials concerned.

As previously remarked, the spectrographic plates employed differed somewhat in development, and possibly also in regard to the intensity (more specifically, number of sparks per second; the nature of each was probably constant) of the analyzer spark during the period in which they were taken. It was desirable to correct for these influences as much as possible before making numerical estimates. From consideration of the probable spark phenomena, and of the behavior of the characteristic (density-versus-intensity) curves for the photographic plates with varying degrees of development, it seemed plausible to fix upon the density of some particular portion of the airband system, averaged for all the spectra on a plate, as a means of such correction. An airband density was found, then, for each plate. The mean for all the plates concerned was determined. This mean value divided by the value for a particular plate gave a factor by which all line densities for that plate were multiplied. These factors were applied to give the corrected, and somewhat ab-

breviated, set of line densities shown in Table II, columns 1, 2 and 3.

The factors ranged from 0.77 for the plate with the M and F spectra to 1.31 for the N 1 to N 5 plate. No great claims are made for the precise accuracy of these corrections, but it is hardly to be doubted that they are in the right direction: they work proportionate increases in line densities for the fainter plates and proportionate

TABLE II

*Mean Corrected Line Densities for Spectra of Leper and Normal Tissues, and Computed Element Ratios. The Ratios Marked \* were Determined Chemically*

Case	Corrected line densities			P/Ca		Na/Ca	
	P	Na	Ca	I <sub>1</sub>	R <sub>1</sub>	I <sub>2</sub>	R <sub>2</sub>
Leper L 11 .....	0.66	0.88	0.76	-0.10	12.00	0.12	11.50
L 12 .....	0.69	0.86	0.59	0.10	30.90	0.27	21.40
L 13 .....	0.70	0.94	0.74	-0.04	15.80	0.20	16.20
L 14 .....	0.68	0.85	0.60	0.08	28.20	0.25	20.00
L 15 .....	0.79	0.91	0.72	0.07	26.90	0.19	15.50
Average .....					22.70		16.90
Normal average							
N <sub>1</sub> -N <sub>5</sub> .....	0.47	0.99	0.96	-0.49	1.90	0.03	7.95
Normal M .....	0.55	0.80	0.66	-0.11	*11.30	0.14	14.10*
F .....	0.52	0.83	0.72	-0.20	* 7.55	0.11	10.50*
A .....	0.44	1.06	0.82	-0.38	* 3.25	0.24	18.10*
Average .....					3.95		10.30
Leper L 3 .....	0.85	0.90	0.94	-0.09	12.60	-0.04	5.90
L S 3 .....	0.57	0.73	0.92	-0.35	3.70	-0.19	3.00
Column .....	1	2	3	4	5	6	7

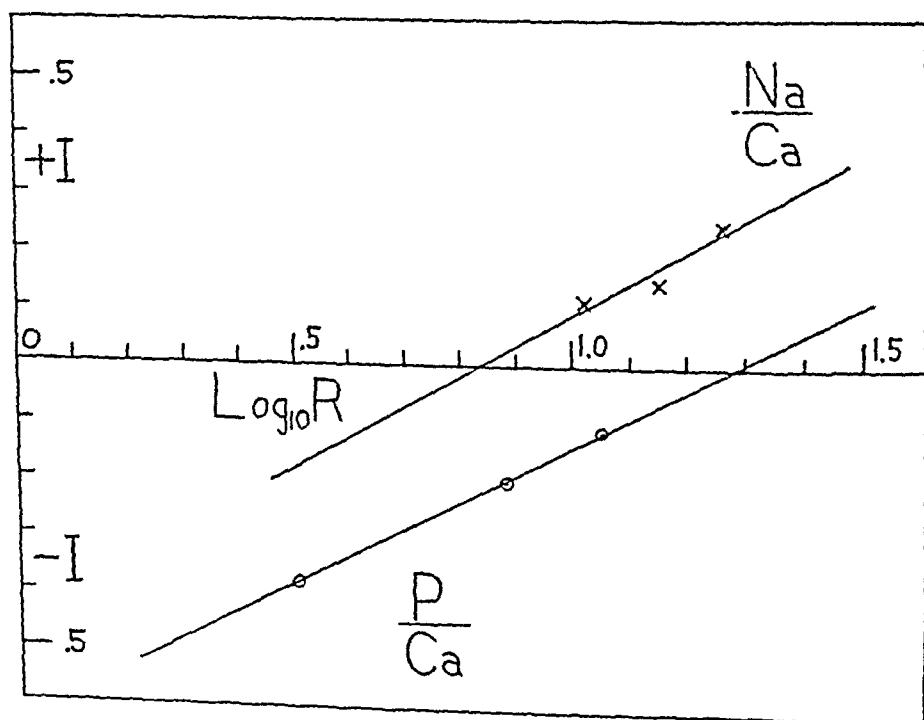
decreases for the blacker ones. Furthermore, the general quantitative estimates are unaltered by the corrections.

Column 4, Table II, gives the differences between the P and Ca corrected line densities for the various materials, which are taken as indices I<sub>1</sub> for the P/Ca ratios R<sub>1</sub> of the materials. For three of these materials (M, F and A) we know R<sub>1</sub> from chemical measurements. Now, to a rough approximation, spectral line density is proportional to the logarithm of the intensity of the light producing the line; and, for minute quantities of an element, the intensity should be proportional to the amount of the element entering the spark. Thus, to get



a curve for purposes of computation,  $I_1$  is plotted against  $\log_{10} R_1$  in Graph 1 for M, F and A, the points being shown as circles in the graph. A straight line is drawn as nearly through the three points as possible. Then the  $R_1$  values for the other materials are secured by fitting their  $I_1$  values to this straight line. For instance,  $I_1$  for leper Case 11 is  $-0.10$ ; the corresponding  $\log_{10} R_1$  value is given by the graph as 1.08, and the antilog of this is  $12.0 = R_1 = P/Ca$  for Case 11. The other cases are handled in the same way.

The indices  $I_2$  for Na/Ca are given in column 6, Table II; and their  $R_2 = \frac{Na}{Ca}$  values are computed as above from the upper Na/Ca line in Graph 1, and are given in column 7, Table II. The crosses are the points from cases M, F and A, which establish the line.



GRAPH 1. Differences (I) in corrected line densities in terms of element ratios (R).

For obvious graphical reasons an R value is likely to be most accurately determined when the degree of extrapolation is least, *i.e.* when its I values fall within the range covered by those of cases M, F and A. The corresponding R ranges for these 3 cases are about 3-12 for P/Ca, and 10-19 for Na/Ca; so, in general, more re-

liance should be placed on computed  $R$  values within, or close to, these limits than on values far outside them. However, the  $P/Ca$  calibration points are sufficiently separated to permit plausible extrapolation for, perhaps, all of the  $R_1$  values given.

## DISCUSSION

Herman Brown<sup>9</sup> has determined, among other things,  $P$ ,  $Na$  and  $Ca$  for a series of normal human skins by chemical means. The samples were taken at autopsy, freed from subcutaneous fat by scraping and cover an age range from the fetal stage to 82 years. The location is not given. There are other figures on  $Na$  and  $Ca$  by the same author in an earlier paper<sup>10</sup> for skin "from the ventral region between the clavicles and the symphysis pubis." The ratio  $P/Ca$  from his figures ranges from 2.6 to 5.5 for the age group 60 to 80 years; our Table II gives 3.95 for the mean of the eight normals. For  $Na/Ca$  in the same age group Brown's figures range from 9.2 to 11.7; Table II gives 10.3 for the normal average. The  $P/Ca$  (but not the  $Na/Ca$ ) for  $L S 3$  also agrees with these chemical results.

These agreements with previously published findings fortify our  $P/Ca$  and  $Na/Ca$  results for leper Cases  $L 11$  through  $L 15$ . The mean  $P/Ca$  for the 5 cases is 22.7; Brown's normal results for the age group (30 to 42 years) concerned range from 4.6 to 6.8 and average about 6.0. We may also note that  $22.7/6.0 = 3.8$  happens to be comparable with  $12.6/3.7 = 3.4$  for  $L 3$  and  $L S 3$ . Thus it seems safe to conclude that the  $P/Ca$  ratio for the cases at hand is around three times higher in well developed leper lesions than in normal skin. As to  $Na/Ca$ , the average 16.9 for the leper lesions is within the range for the corresponding age group as found by Brown (12.0 to 20.0, average around 14.5). It should be noted that the small difference in  $Na/Ca$  ratios mentioned in (B), and further shown in column 7, Table II, was real enough for the samples at hand; it simply becomes of no consequence when the large variation of  $Na/Ca$  with age, as established by Brown's figures, is taken into account.

Consideration of the location of the leprous lesions brings in, however, a factor which we have been unable to check. In the five biopsies they were removed from the ear lobule, forearm, forehead, nape of the neck, and face. These are all exposed parts of the body in contrast with the areas ordinarily covered with clothes, from which we

removed our supposedly normal skin samples. We have not been able to find any data in the literature as to the presence or absence of a difference in the P/Ca ratio of exposed and unexposed parts of the body under normal conditions. Our own attempts to secure samples of normal skin at autopsy from the same exposed areas for spectrographic analyses have not been successful because of the mutilation involved in collecting them. But we think it very unlikely that the high P/Ca ratio in our leprosy cases is due primarily to a regional difference in chemical composition of the skin.

The available evidence points to the conclusion that the deviation from the normal of the ratio is related to the length of time which elapsed since the leprosy condition was first diagnosed clinically. Reference to Table II, column 5, shows that this ratio is high in L 12, 14 and 15 in which the disease had been established for 10, 19 and 8 years and lower in L 11 and 13 of 4 and 5 years duration. But this may not mean so much because there is no assurance that the particular lesions studied were the first detected. In other words, those with unusually high P/Ca ratios may have developed comparatively recently in persons in which the disease had already been well established elsewhere. Brown has found considerable variation in ratio values for undiseased skins from cases of approximately the same age. However, an accidental correlation due to natural variability is improbable where 5 cases are concerned even though the correlation does not involve, for instance, a direct proportionality between ratio and duration.

A high P/Ca ratio signifies either an increase in P relative to Ca, or a decrease in Ca relative to P, or both. We accordingly attempted to discover whether a correlation exists between a high P/Ca ratio and a high percentage in volume of fatty aveolar tissue, sebaceous glands and true leprosy nodule consisting of cells charged with bacilli or with products of their degeneration (foam cells) as compared with other components making up most of the remaining bulk of the skin, namely: epidermis, hair follicles, sweat glands, connective tissue, blood vessels and tissue fluid all grouped together.

Obviously the calculations leave much to be desired from the point of view of accuracy for they were based entirely on the impressions gained by repeated microscopic comparisons of the tissues. Another consideration must be mentioned that may detract from their value. The fifth piece of tissue, into which each specimen removed at bi-

opsy was divided, was frozen and again subdivided, part being used for spectrographic analysis and part for further histological control. The last named was not so well preserved for histological examinations as the four others intentionally fixed for this purpose and, since in L 14 and 15 there was a qualitative difference in the pieces, as already described, it is possible that the tissue burned was not always absolutely comparable to those on which our histopathological account is based.

However this may be, L 12 with highest P/Ca ratio (30.9) showed no fatty areolar tissue; whereas L 11, with lowest P/Ca ratio (12.0), had more than L 15; while L 13 and 14 possessed no demonstrable fatty areolar tissue. Lack of correlation with volume of sebaceous glands was likewise evident. No sebaceous glands were found in tissue from L 12. These structures were most marked in L 11. But the relative volume of leprous cells was noticeably greater in L 12 with the highest ratio than in L 11 with the smallest one. Moreover, in L 11 the bacilli were less numerous and giant globi less conspicuous than in other members of the series. The other cases (L 13 and 14) were difficult to grade and showed no definite correlation of the same sort. Our findings in tissues of L 3, which had to be kept separate from the others because they alone of the leprous tissues were removed at autopsy, suggest, however, the same correlation because the ratio was higher in L 3 with large deep nodules than in L S 3 with smaller ones.

Unfortunately there are no data in the literature on the actual richness in P of leprous nodules of small size dissected free of surrounding tissue. It is merely an assumption that they contain much P and that our correlation between large total volume of cells containing bacilli and their products and high P/Ca ratio partly explains the height of the ratio. We have examined frozen and alcohol-formalin-fixed specimens from the 5 cases by the method of micro-incineration, which is not useful for the demonstration of P, but reveals many mineral constituents including Ca, and have observed that the mineral residue left by the leprous cells is not very extensive. In blood serum Wooley and Ross<sup>11</sup> report, on the basis of chemical analyses, total amounts of Ca and inorganic P which average, for 47 cases of leprosy, well within normal limits as represented by 15 controls. The diffusible Ca, however, in 53 cases averaged considerably lower than in the 15 controls. In a later paper<sup>12</sup> these au-

thors mention the probability that "diffusible calcium" and "available calcium" are essentially synonymous, and advance the opinion that nerve, muscular and bone changes in leprosy may be in part due to this effective Ca deficiency. Perhaps Ca starvation of tissue may be aggravated in the actual foci of leprosy infection and may contribute to the abnormally high P/Ca ratios that we have observed.

The absence in our results of a pronounced difference between leper and normal tissue in regard to Mg/Ca ratio is not surprising in view of the considerable chemical similarity of Ca and Mg. That is, a disease condition tending, say, to reduce Ca content might for this reason have an effect at least in the same direction on Mg. As to the Fe/Ca ratios, it might at first sight seem logically unsound to discount the high Fe/Ca ratio in leper tissue as compared with the normals simply on account of the difference in the way samples were taken and because of the behavior of one leper case (L 3).

#### SUMMARY AND CONCLUSIONS

1. The P/Ca ratios in 5 leper cases studied are on the average probably three times those in normal skins from the same age group. The Na/Ca, Mg/Ca and Fe/Ca ratios show no notable variations from the normal.

2. A fair correlation is obtained in the 5 leper cases for P/Ca ratio with known duration of disease and volume of leprosy cells in tissue analyzed spectrographically. It may be conditioned by increase in P, decrease in Ca, but probably by both.

3. The method of histospectrography, as developed by Scott and his collaborators, can evidently be used for the study of small pieces of tissue removed at biopsy which would be altogether insufficient in amount to permit of routine chemical analysis by ordinary methods. Once the spectrograms have been taken, essentially the same procedure is employed for the determination of ratios between several elements; whereas the chemical estimation of each element would be different and in some cases very involved.

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## DESCRIPTION OF PLATES

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### PLATE 2

FIG. 1. Representative spectra (enlarged) of leprous tissues. The numbers of the cases are indicated in the margin and the lines measured for the particular elements studied are identified below. The two groups of spectra shown for L 11 to L 15 are reproduced from different plates. The spectra from Case 3 (L = heavy lesion and L S = light lesion) are from still another plate. The particular Mg line indicated is visible only with difficulty in these prints. It is immediately to the right of the two very bright lines.

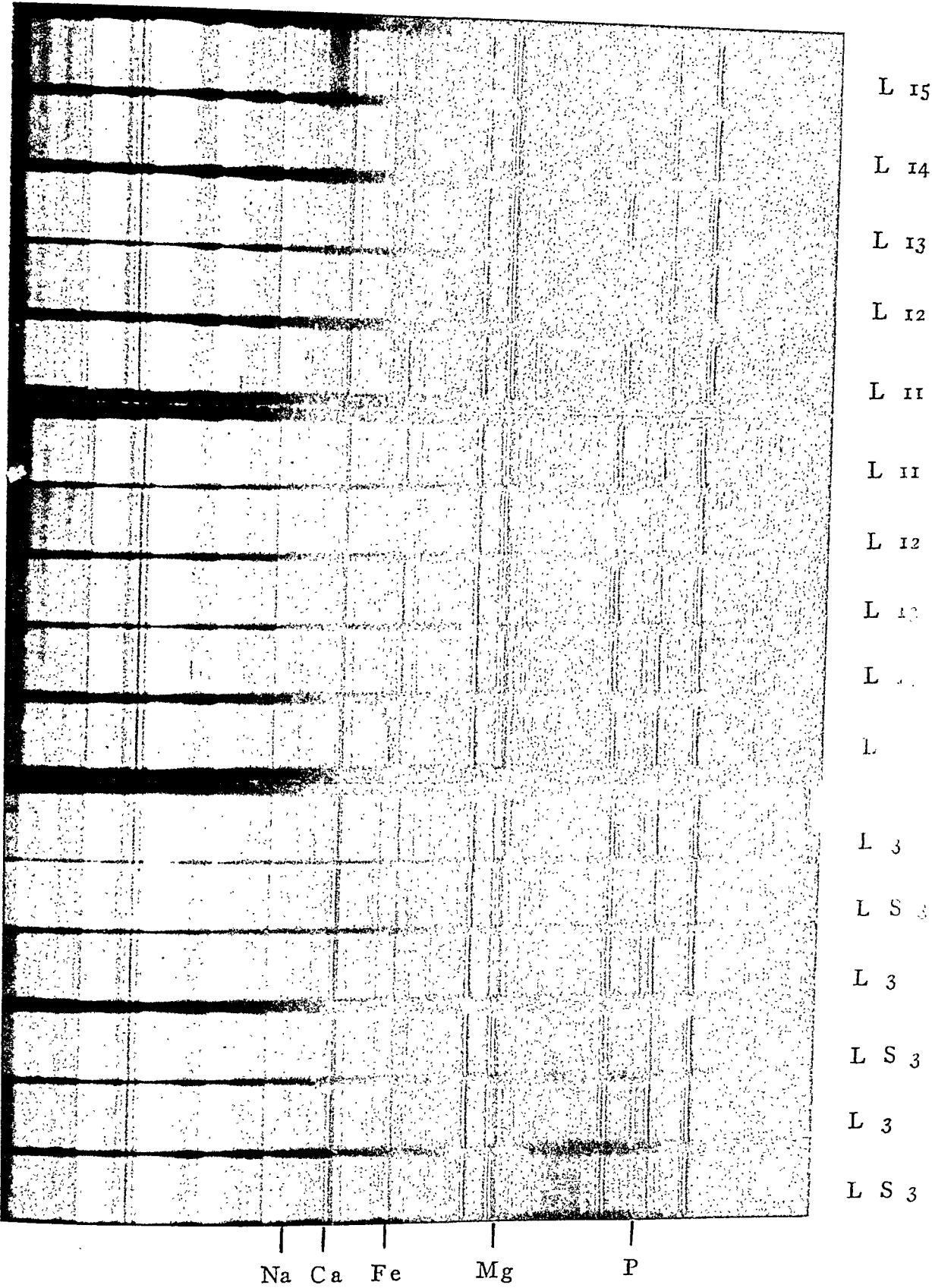
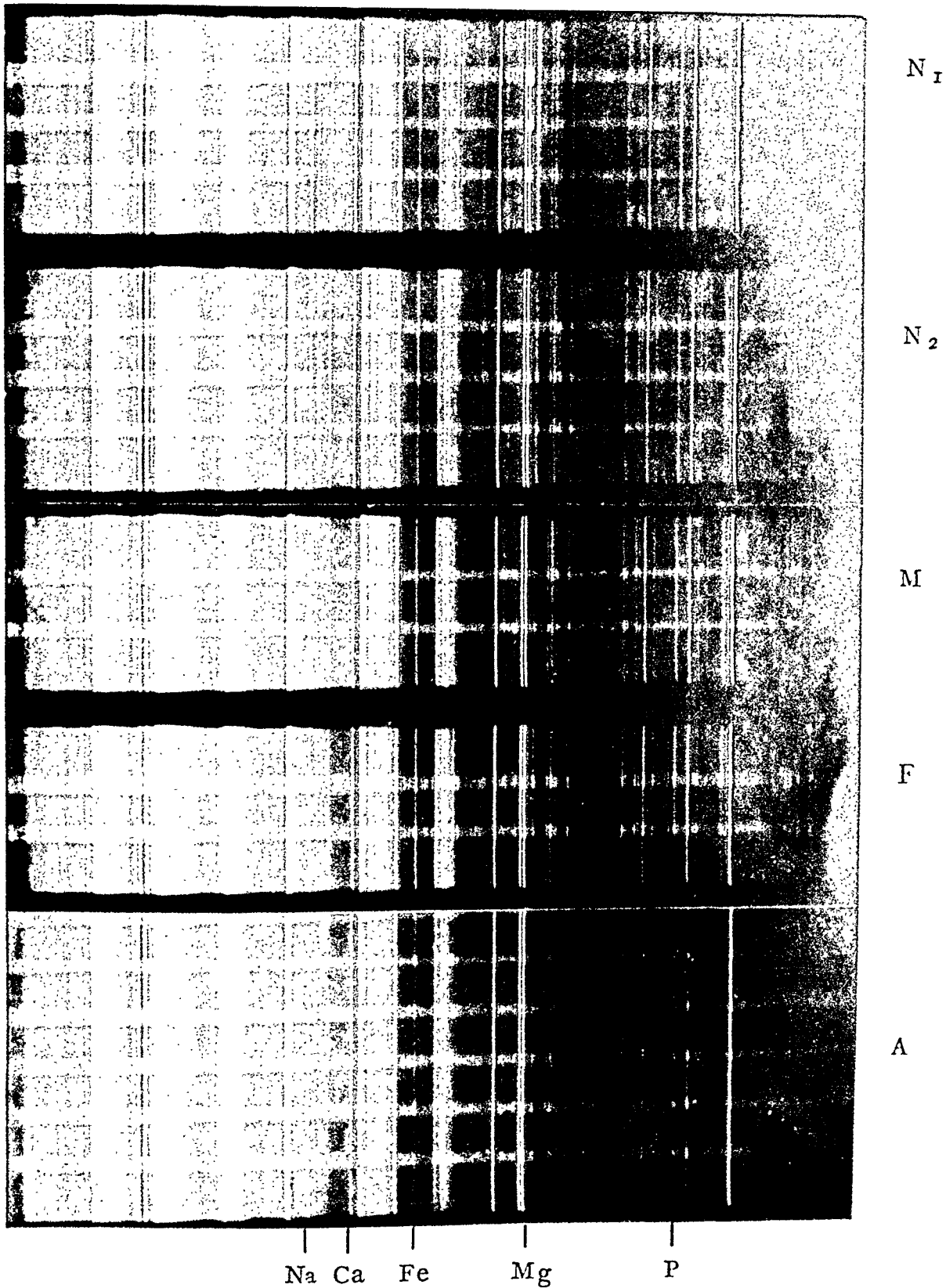




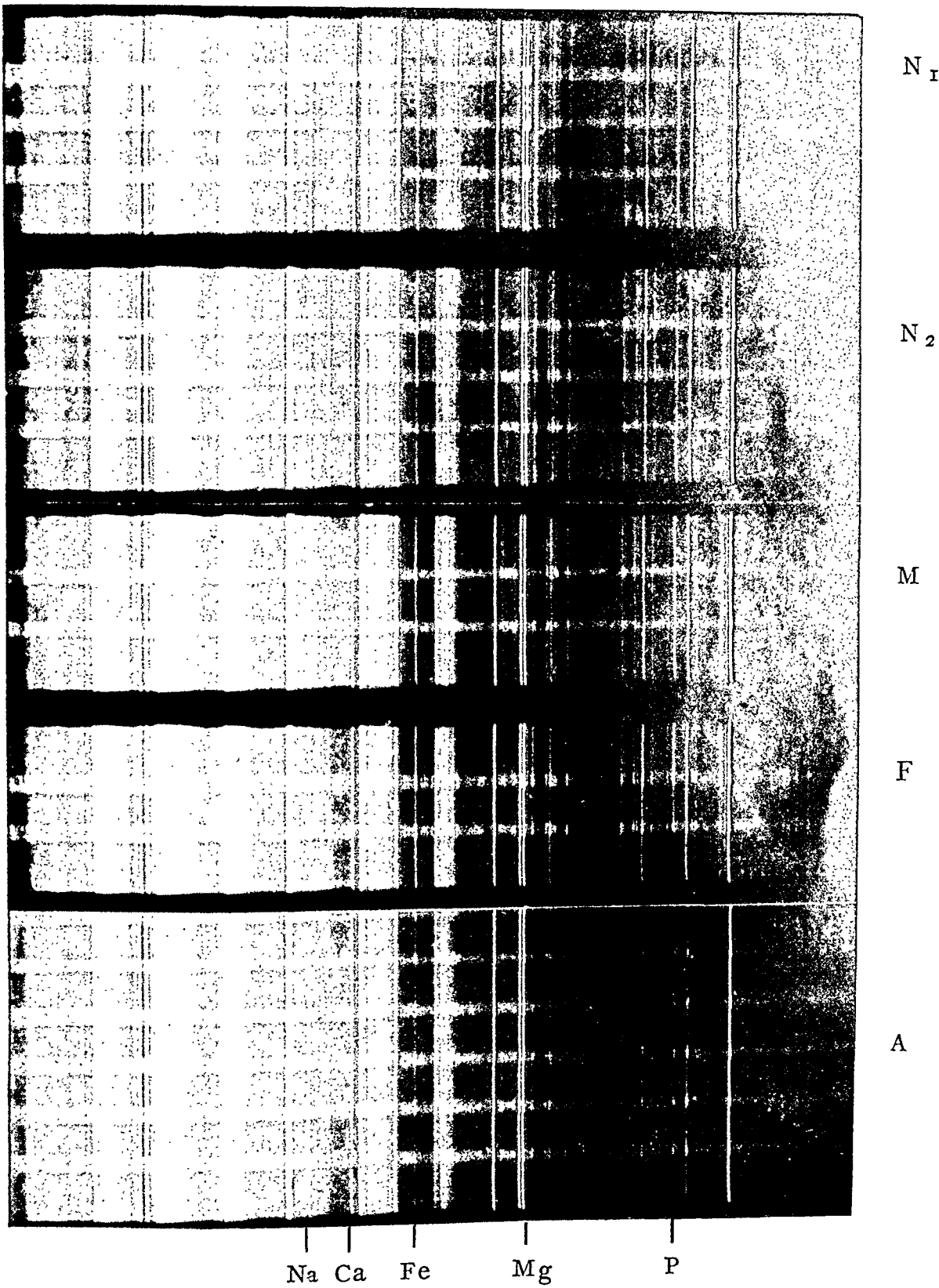
PLATE 3

FIG. 2. Representative spectra of normal tissues for visual comparison with the leprous ones illustrated in Figure 1. Case and line designations are arranged as in Figure 1. N 1 and N 2 are from one plate, M and F from another and A from a third. Note that the phosphorus lines, relative to the calcium lines, are weaker than in Figure 1. This is in accordance with the conclusion reached in the text that the Ca : P ratio is less in leper lesions than in normal tissue.



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# LESIONS IN THE AURICULOVENTRICULAR CONDUCTION SYSTEM OCCURRING IN RHEUMATIC FEVER \*

LOUIS GROSS, M.D., AND B. M. FRIED, M.D.

*(From the Laboratories of The Mount Sinai Hospital, New York City)*

In spite of the fact that disturbances in conduction and rhythm in the heart form an almost invariable and conspicuous finding in active rheumatic fever, remarkably few histological studies have been reported on the tissue of the conduction system in this disease. Thus, for example, the microscopic findings in this tissue from cases of conduction disturbances in rheumatic fever are reported only in single instances each in the excellent monographs of Mönckeberg<sup>1</sup> and Mahaim.<sup>2</sup> In addition to these two authors, scattered reports on very limited material have been made by Gerhardt,<sup>3</sup> Bramwell,<sup>4</sup> Löw,<sup>5</sup> and Naish and Kennedy.<sup>6</sup> The reported findings refer to infiltrations of the bundle with inflammatory cells (in one instance with giant cells), swelling of collagen, "fibrous degeneration of the auriculo-ventricular bundle of His with the presence of a calcareous nodule almost obliterating the bundle," and, more significantly, "lymphocytic infiltration in the region of the node and trunks."

Because of the very limited nature of these reports it is not surprising that various explanations of the cause of the conduction disturbances have been advanced, such as myocardial fatigue, toxic injury, the presence of specific lesions and accumulation of fluid in the synovial sac which was assumed to surround the conduction system in man.

Published reports by one of us (L. G.) with collaborators<sup>7-12</sup> have indicated a high incidence of inflammatory and vascular changes occurring in various parts of the heart in rheumatic fever. It seemed of interest, therefore, to study systematically the conduction system in a representatively large series of cases of unquestionable rheumatic nature in which other affections which might implicate this tissue could be reasonably ruled out.

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Received for publication July 24, 1935.



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## MATERIAL AND METHODS

The findings in 110 hearts form the basis of this report. Of these, 60 presented active rheumatic fever as manifested by the presence of fibrinous pericarditis, acute verrucous endocarditis, Aschoff bodies, eosinophilic swelling of the collagen and other inflammatory changes of the myocardium. Twenty-five cases represented inactive rheumatic material according to the specifications laid down by Rothchild, Kugel and Gross.<sup>13</sup> These cases showed no Aschoff bodies in the myocardium. The remaining 25 cases formed a non-rheumatic control series representing various age periods from birth to the ninth decade of life. This series was carefully studied to eliminate past or present hypertension, subacute bacterial endocarditis and syphilis. A careful study of the clinical records and pathological specimens was made in order to correlate, if possible, the findings with the clinical course of the disease. Electrocardiographic records were studied, when available, with the same object in mind.

The technical methods used were essentially those previously described by Gross and Ehrlich.<sup>7</sup> We have found that a carefully differentiated hematoxylin and eosin stain, as well as a combination of Weigert's elastica and Van Gieson's connective tissue stains, is quite satisfactory for the study of the pathology of the conduction system. The lighter staining of the neuromuscular apparatus, together with its richer vascularity, heavier elastic and collagenous framework and peculiar architectural and topographical relations, render the identification of the specific tissue relatively simple. A variety of stains considered more selective for histological studies of this region is listed by Todd.<sup>14</sup>

Since the studies herein reported are based on an examination of the routine tricuspid valve section obtained by the use of the standardized method of Gross, Antopol and Sacks,<sup>15</sup> the description of the pathological material will concern itself only with the auriculoventricular nodal tissue and the bundle of His as far as and at times including the division into the ventricular limbs. For the sake of brevity this horizontal auriculoventricular conduction system will be referred to as "the bundle." No attempt will be made in this report to describe the findings in the auricular and ventricular ramifications of the Purkinje system since these appear to be considerably less important in the mechanism concerned with the usual conduction disturbances occurring in rheumatic fever.

## TOPOGRAPHY OF THE HORIZONTAL AURICULOVENTRICULAR CONDUCTION SYSTEM IN THE HUMAN HEART

Studies on the histology and topography of the conduction system in the normal human heart have been far more extensive and complete than those on rheumatic hearts. Excellent descriptions are available from the works of His,<sup>16</sup> Tawara,<sup>17</sup> Mönckeberg,<sup>1</sup> De Witt,<sup>18</sup> Tandler,<sup>19</sup> Van der Stricht and Todd,<sup>20</sup> Clerc,<sup>21</sup> Géraudel,<sup>22</sup> Mahaim,<sup>2</sup> Taussig<sup>23</sup> and Todd.<sup>14</sup> For purposes of clarity it seems advisable to present a short description of the Tawara node and the bundle of His \* up to its divisions into the ventricular limbs. This description, which is based on our own observations, will emphasize certain points which will be pertinent to our discussions of the findings in rheumatic fever.

The actual site of the horizontal auriculoventricular conduction system (node of Tawara and bundle of His) with respect to the surrounding tissues shows a moderate amount of variation. There are, however, certain general rules concerning this site and particularly concerning the modification in the histology of the specific tissue as one proceeds from the posterior origin of the system toward its anterior termination into the left and right ventricular limbs. The nodal tissue, or posterior end of the horizontal conduction apparatus, starts in the neighborhood of the coronary sinus ostium as a somewhat loose structure which is either applied very closely to the sub-endocardial tissue of the right auricle or is covered by a wedge of auricular myocardium of varying thickness (Fig. 1). At this point the base of the tricuspid valve generally lies approximately 6-7 mm. below the crest of the septum in the adult heart, and the conduction system on cross-section slopes toward the right from above downward.

The cells themselves may be closely intermingled with the auricular myocardial fibers and merge imperceptibly with them. They are thin, generally running parallel with the auricular fibers in this region, but they may have a whorled architecture. At this site either the bundle itself or the collagenous extension of the septum fibrosum immediately adjacent to the bundle tissue generally contains large

\* Todd does not believe that the auriculoventricular node exists as a special formation. Certainly the structure of the specialized tissue which makes up the so-called Tawara node merges imperceptibly with the more anterior portions of the auriculoventricular conduction system (bundle of His).

blood vessels. Indeed, it is the presence of these large blood vessels within the bundle or in its immediate environs that aids in the identification of the tissue as being nodal or representing the first portion of the bundle of His. Occasionally, at this site, fat cells are intermingled with the nodal tissue. These are sometimes also distributed throughout the auricular myocardial wedge and, contrary to the view held by Engel,<sup>24</sup> apparently do not necessarily represent signs of damage.

In this posterior portion of the horizontal conduction system the left border of the bundle lies against the dense collagenous extension of the septum fibrosum and the outlines of the bundle are extremely irregular. Approximately 4 mm. anterior to this site the bundle is generally more compact, tending to take a roughly triangular form on cross-section with the apex of the triangle cephalad, with one angle sloping somewhat lower toward the right ventricle and the third angle at a higher level sloping toward the left ventricle. The outlines of this portion of the bundle as a whole are generally more clearly defined although still somewhat irregular. The bundle continues to occupy a position to the right of the collagenous septum fibrosum extension; it is somewhat higher placed, with respect to the crest of the interventricular septum, and almost invariably has a large portion of the right auricular myocardial wedge clothing its right face. The left face of the bundle lies against the dense collagenous extension of the septum fibrosum. Below the oblique border of the bundle triangle, and separating it from the crest of the interventricular septum, there is generally a strand of collagenous tissue of varying thickness. The cells of the bundle are generally narrow or medium sized and may either run obliquely or be arranged in whorls. At this site large and medium sized vessels are still frequently found and, not infrequently, fat cells may be irregularly dispersed throughout the bundle or be more aggregated on its right oblique face, thus forming an irregular barrier between the bundle and the auricular myocardial wedge.

Several millimeters anterior to this site (a point at which the base of the tricuspid leaflet is approximately level with the upper border of the septal crest) the bundle tends to take a roughly cylindrical or oval shape on cross-section (Fig. 2). It now occupies approximately the middle of the septum fibrosum, consists of generally large cells, fairly compact, running anteroposteriorly for the most part, and ar-

ranged in basket or bundle form. The whorled appearance has now disappeared. In place of large vessels there are often seen arterioles. Fat cells are infrequent and the collagenous band of the septum fibrosum surrounds the bundle on all its aspects.

At a variable distance anterior to this site (3-5 mm.) the bundle begins to fork into its right ventricular and left ventricular limbs. On cross-section the bundle is generally represented by a somewhat flattened triangle. The cells are large, again beginning to take on an oblique or longitudinal direction parallel with the crest of the interventricular septum and with its walls, the basket arrangement of the fibers may or may not be present, the cells are fairly compact, and dense collagenous tissue surrounds this flattened triangle with its beginning forking of the limbs. If one traces these forked limbs toward the right and left ventricles either at this site or somewhat anteriorly to it, one sees a rapid transformation of the cells into the very large, pale Purkinje fibers. Fat cells are infrequently found in the bundle at this site and the vessels are practically all capillaries.

As a general rule it may be stated that the concentration of elastic and collagenous framework is generally greater in the more posterior aspects of the bundle, chiefly at the nodal site, and becomes increasingly sparse as one progresses anteriorly. The elastic tissue, when present, tends to be concentrated around the blood vessels. The more anterior aspects of the bundle can also be determined by the fact that the right auricular wedge has completely disappeared and the septum fibrosum consists of a strong band of collagenous tissue clothed by endocardium on both sides. The human horizontal conduction apparatus apparently is very poorly supplied by lymphatics. These do not appear to have any predilection site with respect to the location of the section. Vascular channels with prominent endothelial cells are often seen in various portions of the bundle. These may represent collapsed lymphatics or veins.

#### HISTOLOGICAL FINDINGS IN NON-RHEUMATIC CONTROL CASES

As previously stated, the histological studies herein reported are based on an examination of the routine tricuspid valve section (T. V.) obtained by the use of the standardized method of Gross, Antopol and Sacks. This section generally includes that part of the horizontal auriculoventricular conduction system which lies just be-

hind the cylindrical portion of the bundle of His. At times, however, the section was taken either anterior or posterior to this site. In making comparisons of the bundle tissue in the various cases the above described differences in topography, vascularity and histology of these sites were borne in mind.

In order to establish a base line for comparison it is necessary to state briefly the findings in the bundles of 25 non-rheumatic hearts which were obtained at autopsy from patients dying of a variety of diseases other than those which implicate the endocardium. Many of these diseases were of an infectious nature (generally bronchopneumonia). Some were associated with a bacteremia. In the sections studied no appreciable abnormalities were noted either in the distribution or structure of the specific cells of the horizontal conduction system. As was previously indicated, the interstitial framework between the cells of the specific tissue varies somewhat with the site from which the tissue was obtained. None of the non-rheumatic control cases showed a conspicuous increase in this fibro-elastic framework. Only 1 case (a child, 9 years old, dying of a *Staphylococcus aureus* bacteremia following osteomyelitis) showed edema of the bundle and a very definite increase in the interstitial elastic fibers. Apropos of the latter, it may be stated that elastic fibers are either very delicate or non-existent until the latter part of the third or the beginning of the fourth decade of life, after which they become increasingly conspicuous, particularly in the posterior portions of the horizontal auriculoventricular conduction system (nodal portion). Elastic fibers, when present, tend to be concentrated around blood vessels. The collagenous framework between the cells of the bundle is extremely delicate and becomes somewhat more plentiful only from the fifth decade of life on.

During the first four decades of life a very sparse scattering of lymphocytes, occasional macrophages, plasma cells and other mononuclear cells may be seen between the cells of the bundle. Whether these occur in strictly normal material it is difficult at present to state. In making comparisons with the rheumatic material these cells of inflammatory origin will be considered as increased only when they are definitely more numerous than those noted in this control material.

With regard to the histology of the blood vessels in the bundle, as observed in control material, it may be stated that intimal elastifica-

tion is seen in approximately half the cases from the fourth decade on. The capillaries were found dilated in approximately one-third of the cases and dilated lymphatics were seen in approximately one-sixth of the cases.

In view of the findings to be presented in the rheumatic material, it is of considerable interest to note that the collagenous extension of the septum fibrosum abutting against the bundle showed a few blood vessels in 6 of the 25 non-rheumatic control cases. Scattered lymphocytes were exceedingly rare at this site. Elastification of the septum was inconspicuous in this control material.

### HISTOLOGICAL FINDINGS IN CASES OF RHEUMATIC FEVER

Included in the 60 cases of active rheumatic fever were patients who died during what appeared to be the first attack of rheumatic fever, those in which there were repeated attacks previous to the fatal outcome, and patients with chronic valvular disease dying of heart failure whose hearts showed macroscopic and microscopic evidence of activity. All of the 60 cases showed Aschoff bodies in the myocardium. Only 2 of the 60 showed Aschoff bodies in the bundle tissue (Fig. 3). These were both from patients dying during a first attack. Evidently, therefore, this rather rare presence of Aschoff bodies is not sufficient to account for the frequent conduction disturbances during life.

Of greater frequency was the accumulation of inflammatory cells in the bundle (Figs. 4, 5 and 6). This occurred in 13 cases (22 per cent) to an extent greater than that met with in the control material. The preponderating cell was generally the lymphocyte. Occasionally, polymorphonuclear leukocytes were the more conspicuous of the cellular elements. Together with these there were noted plasma cells, macrophages and, at times, young fibroblasts, although these occurred in much smaller proportions. The cells were generally irregularly distributed throughout the bundle tissue, but tended to be more concentrated at the borders of the bundle as it abuts against the adjacent tissue.

Edema of the bundle, which is represented by the appearance of sometimes lightly basophilic material lying in the interstices between the cells, was found in 9 cases (15 per cent). This was found only in the patients dying during the first attack (Figs. 3 and 4). As regards the supporting framework of the bundle, there did not appear to be

any increase in collagen in the cases falling into this group. On the other hand, the elastic tissue framework appeared to be definitely increased in 22 cases (37 per cent). This was noted even after making due allowance for variations in the site of the bundle, as well as for age periods. In the majority of instances this increase in elastic tissue was found in the cases of chronic valvular disease where the individual died in decompensation but still showed active rheumatic disease (Aschoff bodies, and so on) histologically.

Considered as a whole, exudative phenomena were found in some portions of the bundle during the active phases of rheumatic fever in over one-third of the cases. Serial sections would undoubtedly have shown a higher incidence. It may be stated that the greatest increase in the incidence of inflammatory phenomena, as well as in their extent, was noted in the clinically very active cases. As is well known, conduction disturbances are much more likely to take place in these very active cases.

A very striking finding in the cases of active rheumatic fever was the presence of a variety of vascular lesions. These lesions were qualitatively similar to a number of those described as occurring in the myocardial coronary tree in active cases of rheumatic fever.<sup>10</sup> Thus, 15 of the 60 cases (25 per cent) showed intimal musculo-elastic hyperplastic changes (Fig. 5). As mentioned in previous reports, this lesion is highly characteristic, if indeed not specific, of rheumatic fever. Twenty cases (33 per cent) showed intimal proliferation with minimal or no elastic changes. Two cases showed thrombi lying in small veins. In 1 case the smooth muscle nuclei showed vacuolization. Three cases showed marked medial hypertrophy. Many cases showed dilated capillaries and some showed thickened ones.

Considered as a whole, these vascular changes occurred much more conspicuously and frequently than the exudative phenomena. To this must be added the fact that not all the cases studied were represented by a section through the posterior portion of the horizontal auriculoventricular conduction system which contains the larger vessels. Had such sections been available for study, it appears likely that approximately twice the number of cases indicated above would have shown these vascular lesions. This would bring the incidence of vascular lesions in general in the active rheumatic cases to approximately 66 per cent, and the incidence of intimal musculo-elastic hyperplastic lesions to about 50 per cent of the cases.

One of the most interesting findings in the active rheumatic material, and one which possibly plays a rôle in the production of conduction disturbances, is the frequent occurrence of inflammatory disturbances in the collagenous extension of the septum fibrosum which abuts against the bundle tissue (Figs. 4 and 6). As previously indicated, this collagenous tissue shows one or two vessels in the proximity of the bundle in approximately 24 per cent of the control non-rheumatic cases. A very rare scattering of lymphocytes is occasionally found near these vessels. In contrast to this, a conspicuous increase in vascularity, as well as in inflammatory cells, was found in 29 cases (48 per cent) of the active rheumatic series. These findings were noted chiefly in the cases where death took place during a first attack, especially in the clinically active cases. The inflammatory cells were similar to those described as occurring in the bundle. In 2 cases the inflammation of the septum was so extraordinarily marked that the collagenous band was converted into a mass of active granulation tissue (Fig. 6). In 2 cases the blood vessels showed intimal musculo-elastic hyperplastic lesions. In 1 case Aschoff bodies were present in the septum. In a significant number of these cases the elastic tissue of the collagenous extension of the septum fibrosum was definitely increased.

The findings in the 25 cases of inactive rheumatic fever, where most of the individuals died of extracardiac causes, are conspicuous by their paucity. These were in all respects similar to those described as occurring in the non-rheumatic control series. Evidently, therefore, the cases of rheumatic fever which are of so mild a nature as to permit of complete clinical and anatomical inactivation of the inflammatory process, leave no appreciable clinical or histological evidence of disturbance in the horizontal auriculoventricular conduction system.

## DISCUSSION

Electrocardiographic tracings were available for study in only 16 of the 60 active rheumatic fever cases. From this limited material it was impossible to establish a direct relation between the extent of the abnormal electrocardiographic findings and the intensity of the inflammatory lesions in the bundle and in the tissue surrounding this structure. In spite of the fact that usually only one or two sections were studied from each case, at least 66 per cent of the cases showed



exudative or vascular lesions within the bundle or within the tissue in its immediate neighborhood. As indicated above, it seems probable that serial sections would have raised the incidence of these lesions to an even higher level.

The finding of the frequent involvement of the collagenous extension of the septum fibrosum appears to be of considerable interest both from the functional point of view as well as from considerations concerning the spread of the infection. Heart block due to contiguity spread of presumptively syphilitic lesions from the root of the aorta to involve the bundle of His and septum fibrosum has been observed by Sohval.<sup>25</sup> The very high incidence of vascular and exudative lesions in the septum fibrosum extension in cases of active rheumatic fever suggests the possibility that these may be due to a contiguity process from the root of the aorta, the ring of the aortic cusps (chiefly the posterior), as well as from the auricular myocardial wedge. This would account for the frequency with which the bundle of His is involved in rheumatic fever and for the functional evidences of this involvement as indicated by the frequent conduction disturbances found in this condition. An added factor in the mechanism of the production of these conduction disturbances may lie in the topographical relations of the bundle. Inasmuch as one part of the conduction system is entirely surrounded by the relatively rigid collagenous tissue, this non-yielding mantle probably limits swelling and expansion of the bundle tissue when this is involved in an exudative process. As a consequence, compression of the cells takes place. There does not appear to be any evidence to support the assumption that edematous fluid within lymphatics may be responsible for these functional changes. Furthermore, as is well known, the horizontal conduction system in the human heart is not surrounded by a lymphatic sheath such as is seen in animals.

The absence of inflammatory and vascular lesions in the group of cases which showed chronic valvular disease without the presence of activity in the myocardium suggests the possibility that many of these processes, if indeed not all of them, may be capable of healing without leaving appreciable characteristic residua. That this is possible in the case of some vascular lesions in rheumatic fever has already been indicated elsewhere.<sup>10</sup> Surprisingly enough, this may also hold true for elastification of the bundle and the collagenous extension of the septum fibrosum, inasmuch as elastification of these sites

is not infrequently encountered in the active cases but is seldom seen in the inactive ones. As indicated before, an alternative hypothesis would be that the chronic valvular disease cases without activity represent a clinical course which was extremely mild even though continued over some period of time, and that eventually complete healing took place. Perhaps under such circumstances involvement of the bundle is minimal, so that one does not have to assume restitution to integrity of advanced lesions.

Finally, it may be stated that in the course of active rheumatic fever there occurs a series of exudative and vascular phenomena which are sufficiently frequent and intense to account for the high incidence of conduction disturbances.

### SUMMARY AND CONCLUSIONS

One hundred and ten human hearts have been examined in order to determine the nature and frequency of the lesions occurring in the Tawara node and bundle of His in rheumatic fever. Sixty of these cases represent active rheumatic fever, 25 cases inactive rheumatic fever, and 25 cases non-rheumatic material. It has been shown that in active rheumatic fever there occurs a variety of inflammatory and vascular phenomena within the horizontal conduction system as well as in the surrounding tissue. Even when studied in few representative specimens from each bundle, the incidence of these lesions was approximately 66 per cent in the active material. It is probable that a study of more sections would have indicated a higher incidence. Very few of these lesions are of a specific or highly characteristic nature. The inactive rheumatic cases showed few pathological changes. This is in keeping with the functional differences observed as between these two groups. Attention has been called to the high incidence of inflammatory lesions in the collagenous extension of the septum fibrosum and a discussion of the possible mechanisms concerned with the spread of the rheumatic infection to the bundle tissue is given. A description of the topographical relations of the horizontal conduction system in the human heart, together with the findings in 25 non-rheumatic control cases is also given.

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## DESCRIPTION OF PLATES

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### PLATE 4

FIG. 1. Cross-section of horizontal conduction system from non-rheumatic control case taken from its posterior portion. Age 31 years. Weigert's elastic and Van Gieson's connective tissue stain. Low power.

A = bundle tissue. Note whorled arrangement of the cells; B = artery of the bundle; C = sparse auricular myocardial fibers lying on right side of bundle; D = collagenous extension of septum fibrosum lying on left side of bundle.

FIG. 2. Cross-section of horizontal conduction system from non-rheumatic control case taken from its middle portion. Age 62 years. Weigert's elastic and Van Gieson's connective tissue stain. Low power.

A = bundle tissue; B = fat cells separating bundle from right side of septum fibrosum; C = left ventricular endocardium covering septum fibrosum.



PLATE 5

FIG. 3. Cross-section of horizontal conduction system from case of active rheumatic fever (first attack). Age 10 years. Hematoxylin and eosin stain. Low power.

A = Aschoff bodies in edematous and inflamed bundle tissue; B = large blood vessels (injected) of bundle showing inflammatory cells in adventitia.

FIG. 4. Cross-section of horizontal conduction system from case of active rheumatic fever (first attack). Age 17 months. Hematoxylin and eosin stain. Low power.

A = inflamed and edematous bundle tissue. Note compression of cells; B = markedly inflamed septum fibrosum. Note marked increase in capillaries and inflammatory cells; C = zone of marked inflammation with arterioles; D = concentration of inflammatory cells between bundle and septum fibrosum.





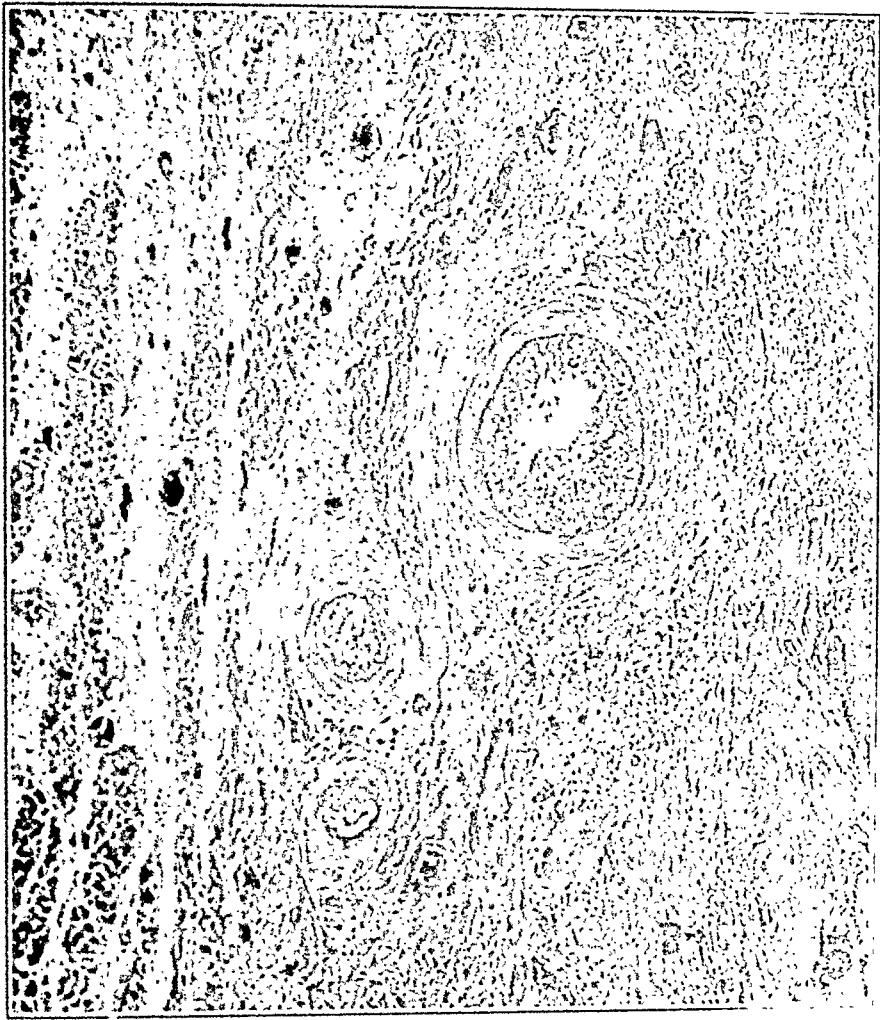
PLATE 6

FIG. 5. Cross-section of horizontal conduction system from case of active rheumatic fever showing typical musculo-elastic hyperplastic vessels. Age 9 years. Weigert's elastic and Van Gieson's connective tissue stain. Low power.

A = marked inflammation with engorged capillaries; B = arteriole in inflamed septum fibrosum.

FIG. 6. Cross-section of horizontal conduction system from case of active rheumatic fever (first attack). Age 17 months. Hematoxylin and eosin stain. Low power.

A = inflamed bundle; B = septum fibrosum permeated with inflammatory cells and capillaries of the granulation tissue type; C = concentration of inflammatory cells between bundle and inflamed septum fibrosum.



Lesions in Conduction System in Rheumatic Fever



# BENIGN AND MALIGNANT HYPERTENSION AND NEPHROSCLEROSIS \*

## A CLINICAL AND PATHOLOGICAL STUDY

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### INTRODUCTION

In the classification of Bright's disease there still exists much confusion. The number of historical reviews on the subject makes it unnecessary to discuss in detail this aspect of the question. Our objective has been a clinical and pathological study of the renal manifestations of arterial hypertension in an endeavor to reach a clearer understanding of the conditions commonly labeled "benign and malignant hypertension or nephrosclerosis."

Owing to the reciprocal relationship of hypertension and kidney disease the later stages of both conditions often present great difficulty in differentiation to both pathologist and clinician. It is, therefore, essential to describe in detail the histological criteria we employed in excluding those renal conditions which produce "secondary hypertension." Disregard of these considerations by many authors is to a great extent responsible for the difficulty in reconciling the various classifications of Bright's disease.

### *Criteria Used for Differential Diagnosis*

The outstanding conditions to be discussed are diffuse glomerulonephritis and ascending processes leading to contraction of the kidney.

(1) *Diffuse Glomerulonephritis*: In making the diagnosis of diffuse glomerulonephritis the most important feature is the diffuseness of the glomerular lesion. In the acute and subacute stages the clinical and histological pictures are sufficiently characteristic to make the diagnosis free from doubt. It is in the chronic stage — the so-

\* Received for publication August 8, 1935.

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called secondary contracted kidney — that difficulties arise. The past history of the patient is in many instances incomplete or reveals no evidence of a preceding attack of acute nephritis. Hypertension with variable albuminuria over a period of years is often the only manifestation. Histologically the glomerular lesion may not be an entirely diffuse one — the percentage of diseased glomeruli frequently not exceeding 60 to 70 per cent. Furthermore, secondary arterial and arteriolar changes may in the later stages complicate the picture to such an extent that it is impossible to decide whether the glomerular or vascular lesion predominates. We have excluded cases of this type for reasons which will be discussed later. The gross appearance of the kidney in diffuse glomerulonephritis, though in many cases characteristic, is subject to such wide variations that it is an unreliable criterion in differential diagnosis. In short, we based the differential diagnosis of glomerulonephritis on a predominantly diffuse glomerular lesion, especially in cases where the clinical data were inconclusive.

(2) *Ascending Contraction of the Kidney:* This often presents still greater difficulty in recognition. Here again the clinical history may afford little or no assistance. Histologically the main diagnostic feature is the interstitial infiltration which is usually, but not invariably, most marked in the medulla. The macroscopic appearance of the kidney may be more characteristic. Widening of the calyces, thickening, hyperemia and dullness of the mucosa may suggest the ascending nature of the process, while the breadth and flatness of the cortical scars indicate that the contraction originated in a large group of collecting tubules, thereby involving a wide zone of renal substance. It has to be emphasized that widening of the renal pelvis is not invariably present and in long-standing cases the gross appearance may be so indefinite that the diagnosis has to be made on histological grounds. Unfortunately, it is in this type of case that secondary vascular changes tend to be most severe and the picture may be so complicated as to make a definite decision impossible. Certain histological features are, however, characteristic. The distribution of the cellular infiltration has been mentioned. Its character is of importance — the presence of plasma cells, monocytes and leukocytes enabling us to discriminate between the inflammatory and urely ischemic scarring processes. Leukocytic cylinders are frequently encountered, particularly in the collecting tubules. Most

remarkable is the relative infrequency of inflammatory changes in the glomeruli. There may be thickening of the glomerular capsule, usually associated with some degree of atrophy, such atrophied glomeruli being characteristically crowded together in scarred areas from which the tubules have disappeared.

A picture closely resembling the above may be encountered in the condition Fahr<sup>1</sup> has termed "incomplete infarction" in which circulatory insufficiency causes atrophy of the tubules while the glomeruli for the most part remain intact. The finding of an old arterial thrombosis or very severe arteriosclerosis in the artery of supply points to the diagnosis, but in the absence of such indication the character of the interstitial infiltration must be taken as a guide. In the resorptive scar tissue of incomplete infarction inflammatory infiltration of the type described above is absent. The whole available evidence—clinical, macroscopic, and microscopic—must therefore be considered in making the exclusion diagnosis of old ascending processes.

We have briefly referred above to examples of extreme contraction of the kidney in which vascular and glomerular lesions are inextricably mixed. To these may be added advanced cases of ascending contraction. All such instances represent the final stages of a disease whose early origin it is impossible to recognize with certainty. They constituted only a very small group in our series and we felt it justifiable to exclude them on the above grounds.

*Benign Hypertension, Benign Nephrosclerosis and Malignant Nephrosclerosis (Fahr<sup>1</sup>)*

We have discussed above the method of exclusion of cases of glomerulonephritis and ascending contraction. The remaining cases appear under a somewhat confusing variety of terms. Histologically the kidneys show all degrees of arterial and arteriolar changes of different types overshadowing any glomerular and tubular lesions which may be present. Clinically renal involvement may or may not be evident. According to Fahr these cases would fall into three groups—essential hypertension, benign nephrosclerosis and malignant nephrosclerosis. Since a critical analysis of this classification has been our special objective, it is desirable to outline the general conceptions involved. As "essential hypertension" Fahr designates cases in which renal vascular changes are absent. When the kidneys show arterial and arteriolar sclerosis the term benign nephrosclerosis

is used and the hypertension is considered to be secondary to the renal vascular changes. The differentiation of essential and benign renal hypertension is therefore made on the basis of a quantitative estimation of the diffuseness of arterial involvement (Fahr<sup>2</sup>). Histologically there is no clear line of demarcation between benign nephrosclerosis and Ziegler's "Arteriosclerotische Schrumpfniere," that is, circulatory atrophy without hypertension, but a presumption in favor of the former may be made on the basis of a diffuse arteriolar sclerosis.

As a subgroup of benign nephrosclerosis Fahr has described a series of cases showing histologically focal glomerulitis. On the basis of these lesions and the presence of elevated non-protein nitrogen in the blood in such cases, Fahr regards this as a decompensated form of benign nephrosclerosis. Other observers (Volhard,<sup>3</sup> Lichtwitz<sup>4</sup>) consider that a true renal decompensation cannot be recognized clinically and maintain that cardiac failure is chiefly responsible for the nitrogen retention (see page 66).

The malignant nephrosclerosis of Fahr is characterized by specific arterial lesions in the kidneys, namely, productive endarteritis and necrotizing arteriolitis. The latter change is considered of greater diagnostic value since the former may occasionally be absent. Focal glomerular lesions are present similar to those found in decompensated benign nephrosclerosis but are usually more severe in character and extent. The tubules commonly show degenerative changes. The hypertension is regarded as secondary to the arterial and arteriolar lesions which in turn are believed to result from the action of an exogenous toxin. Characteristically the lesions are fairly diffuse in the kidney and may be present in other organs, especially those of the splanchnic area. The vascular necrosis with reactive, exudative and proliferative changes resembles in its most marked form the condition known as periarteritis nodosa, though the latter usually affects larger vessels and has a wider organ distribution.

As Volhard points out, it has become an urgent clinical necessity to establish criteria for the differential diagnosis of benign and malignant hypertension. Fahr has given us certain criteria for making the differentiation histologically. The reasons why Volhard and Fahr's conception of malignant nephrosclerosis as a definite disease entity has not received universal acceptance are twofold. First, there exist many borderline or transitional cases which give rise to great diffi-

culties in classification; second, the "specific" arterial changes are frequently encountered in other conditions, such as diffuse glomerulonephritis, where their rôle as primary lesions can obviously not be maintained. To these criticisms we have directed our attention — in particular examining the controversial "borderline" cases from a clinical and histological standpoint. We have come to the conclusion that these cases are of vital importance, not only as a criticism of the classifications presented by Volhard and by Fahr but more especially in giving a clearer conception of the nature and course of the malignant type of hypertension.

### *General Histological Changes*

#### *A. Arteries*

For rough comparative purposes we made a distinction between large, medium-sized and small arteries and studied the lesions in each. In conformity with the majority of observers we considered as small vessels (arterioles) all sizes up to the interlobular arteries. We differ in this respect from Bell and Clawson,<sup>5</sup> who would confine the term arteriole to the vasa afferentia. Arterial changes were studied not only in the kidney but in other organs, especially the pancreas and adrenals.

(1) *Large and Medium Sized Arteries:* Passing over the common form of arteriosclerosis we would call attention to one special form of it, the so-called productive endarteritis, especially to emphasize the difficulties that may arise in differentiating it from pure arteriosclerosis or "elastosis" of Volhard. Between the two, all transitional stages are encountered. In clear-cut cases of productive endarteritis degenerative changes are absent from the media, which tends rather to undergo muscular hypertrophy. The subintima is nucleated and has the appearance of "onion layers." In this pure form there is no difficulty in distinguishing the lesion from fully developed degenerative arteriosclerosis. Secondary degenerative changes may, however, occur in endarteritis, for example, mucoid and hyaline material may appear in the intima, in which case the latter has a less nucleated appearance. There is no clear line of demarcation between this picture and that of a purely degenerative arteriosclerotic change. Since both types of lesion may occur in different stages in the same kidney it may be almost impossible to make a definite decision.



In taking productive endarteritis as a criterion for the provisional diagnosis of malignant nephrosclerosis we accept, therefore, only those cases in which it occurs diffusely or at least in which it is the predominating arterial lesion. Focal endarteritis, especially of the transitional type described above, is a fairly common finding in scarred kidneys and is of no diagnostic value.

(2) *Small Arteries*: It is unnecessary to comment on the common arteriosclerotic changes — hyalinization and fatty degeneration. One point should be emphasized — the frequency of severe arteriosclerosis in the suprarenal glands is given surprisingly little notice in the literature, yet we found that this organ was affected with about the same degree of severity as the pancreas. In the case of the suprarenal the arteriosclerosis is predominant in the arteries of the periadrenal fatty tissue from which the gland receives the blood supply.

We have, furthermore, occasionally encountered a peculiar type of staining reaction with eosin-methylene blue which appears to be of some significance. The arteriolar wall takes on a fairly diffuse bluish appearance contrasting strongly with the bright red hyalinized arterioles. In a severer form the vessel wall may give a homogeneous dark blue stain (Fig. 1), or the dark staining material may appear as cloudy bluish masses, or again as sharply defined flakes (Fig. 2). The latter appearance is particularly common in the periadrenal fatty tissue. The exact significance of this change is uncertain. It is most frequently seen where arteriosclerosis is severe and is often associated with true arteriolonecrosis. We did not feel that it could be regarded as an acute necrosis in Fahr's sense since disintegration of the vessel wall, invasion by red blood cells and exudative and proliferative changes were absent. We termed it "fibrinoid degeneration," concluding that in rate of development and severity it probably occupies an intermediate place between arteriolar necrosis and hyalinization.

The term "necrotizing arteriolitis" (Fahr) is used to bring this type of lesion into contrast with hyalinization of the arterioles, and is employed on account of the frequent association of the arteriolar necrosis with an inflammatory reaction, which is never found in hyalinization.

In small, medium and large arteries respectively, we attempted to make a rough quantitative estimate of the severity of the arteriosclerotic process for comparative purposes. Taking into account the

differences of distribution, as well as the degree of change in the individual vessels, four degrees of severity were arbitrarily recognized for each vessel group and expressed numerically (1-4). The sum of these figures for all three vessel groups is taken as representative of the degree of arteriosclerosis in the kidney under examination.

### *B. Glomeruli*

(1) *Purely Degenerative Changes:* The processes of atrophy and hyalinization of the glomeruli in kidneys showing arterial or arteriolar sclerosis do not require detailed consideration. Their modes of development have recently been reported in a paper by one of us.<sup>6</sup> An occasional finding of some interest is a diffuse and very striking thickening of the intercapillary connective tissue. A detailed study of this condition will be presented in a separate communication.

(2) *Inflammatory Changes:* (Figs. 3-6) Inflammatory lesions of the glomeruli in cases of hypertension associated with renal vascular disease are subject to wide differences in interpretation. In Fahr's malignant nephrosclerosis, glomerulitis, though essentially focal in distribution, may be so severe and extensive that some observers regard it as a primary glomerulonephritis.<sup>21</sup> Cases of benign hypertension showing glomerular changes of this type are much less frequent and the lesions relatively scanty, so much so in fact that occasionally a careful search reveals only two or three affected glomeruli in each section. Their existence, however, is of sufficient importance to merit a detailed description, especially since Fahr emphasizes the proliferative character of the lesion which in our cases has been minimal. The earliest change to be observed is a swelling of the epithelial cells of the glomerular loops. The cells may present a honeycomb appearance which is occasionally attributable to the deposition of fat droplets. The capillary basement membrane may undergo slight degenerative changes, giving it an irregular appearance, and collapse of the capillary makes the affected loop stand out from the remaining patent capillaries of the glomerulus. In other glomeruli the cytoplasm of the enlarged epithelial cells takes on a bluish stain and the nuclei undergo fragmentation. Early fibrinous adhesions to the parietal layer of Bowman's capsule may be present. In later stages there is definite broadening of the capillary wall, which takes on a turbid, finely granular, purplish red appearance with blurring of its

outline. The nuclei become irregular and pyknotic and at the center of the glomerular loop disappear entirely. At the periphery, especially at the site of adhesions, nuclear proliferation may be seen, but increase in polymorphonuclear leukocytes is very rare. Occasionally areas of capillary collapse give the impression of an increase in nuclei, but the phenomenon is more probably a crowding effect. In still later stages the affected loops take on a homogeneous appearance resembling hyalinization which may be focal or may involve the whole glomerulus. This picture is very characteristic and the cosin-methylene blue stain reveals a pinkish, homogeneous hyaline area containing sharply demarcated, flake-like masses which have a vivid red or bluish hue. The fat stain reveals much neutral fat and lipoids in these areas of "hyalin necrosis," and doubly refractile substances are frequently present.

These glomerular lesions are principally of an "alterative" nature but according to the severity of the process may be followed by exudative or proliferative changes in varying degree. Even in the presence of the latter, however, the primary necrotizing process is always evident. On this account we applied the term "alterative glomerulitis" and contrast the lesion with that of acute diffuse glomerulonephritis in which the primary alterative changes can be demonstrated only with difficulty.

Other forms of glomerulitis, such as are produced by embolic processes, acute suppurative changes, periglomerular infiltrations and agonal fibrin thrombi, are occasionally encountered but their presence is so obviously incidental that no further mention is considered necessary.

### *C. Tubules*

The common regressive changes, albuminuric degeneration, hyaline droplet degeneration, necrosis and fat deposition do not demand detailed consideration. Their occurrence is common to all types of arteriosclerotic kidneys, although hyaline droplet degeneration is more frequently seen in the malignant type than in the benign type of nephrosclerosis. The features to which we pay special attention are tubular dilatation and hyperplasia. It is a rather obvious assumption that tubular dilatation is associated with decrease of kidney function, but we have been unable to find any comparative study of the relationship from the clinical and histological aspects.

Jores <sup>7</sup> distinguishes different forms of tubular hyperplasia. Among these are (1) dilatation of the lumen with enlargement of epithelial cells, (2) lateral sprouting and (3) prolongation of tubules — recognizable by the increase in number of tubular cross-sections relative to the number of glomeruli. We confined our observations to types (1) and (2) and recognized four degrees of dilatation — slight, moderate, considerable and severe. The first degree may perhaps be described as questionable; the last, so commonly found in chronic glomerulonephritis, is rarely to be seen in arteriosclerotic contracted kidneys. We realize that this method is only roughly quantitative and open to subjective errors. Nevertheless, it is in our opinion the only reliable morphological guide to functional impairment. The degree of kidney shrinkage in the individual case may be misleading, although the average kidney weight in severe arteriosclerosis is subnormal. Histologically resorptive scar tissue and hyalinized glomeruli may give a false impression of the extent of reduction of kidney parenchyma. The severity of the arteriosclerosis may be an equally unreliable guide to impairment of function, since it has been shown by perfusion experiments (Kimmelstiel <sup>8</sup>) that no exact parallelism exists between the degree of arteriosclerosis and diminution of blood flow through the kidney. Tubular dilatation with hyperplasia, on the other hand, is a direct response to reduction of kidney parenchyma below the functional reserve, and for this reason has received our special attention. According to the rate and distribution of the scarring process tubular dilatation may be focal or more or less diffuse, and this variation has to be considered in estimating its degree of development. It is furthermore a common observation, and one for which we have no adequate explanation, that in early cases tubular dilatation is confined to the periphery of the cortex immediately below the kidney capsule.

### *Clinical Investigations*

(a) *Hypertension*: Elevation of blood pressure was the basis of selection of our cases. As to the duration of the hypertension our information is naturally incomplete in many instances. Moreover, on the final admission to the hospital a number of the cases showed slight or absent elevation on account of heart failure or coronary thrombosis. There was, however, in such cases a well authenticated

previous history of hypertension, or an increased heart weight with left ventricular hypertrophy but without valvular lesion was found at autopsy.

(b) *Renal Function Tests*: Chief emphasis has been placed on urine concentration tests and the level of the non-protein nitrogen of the blood. Volhard's dilution-concentration tests are available in many instances but in their absence repeated routine specific gravity tests have been accepted. Any inaccuracy arising from this source is in a positive direction, that is, values for concentrating power, obtained from repeated specific gravity tests on routine specimens, tend to be higher than those given by the concentration dilution test. Abnormal urinary constituents — albumin, erythrocytes, leukocytes and casts — have been noted. Other tests of renal function, creatinine clearance, urea clearance and the phenolsulphonephthalein tests have been studied, but as these are not available in the majority of cases, they are used only as supporting evidence and have not been employed for comparative purposes.

(c) *General Features*: Particular attention was paid to the age of the patient, past history of renal disease, scarlet fever or toxemia of pregnancy. Details of ophthalmoscopic examination of the retina were obtained whenever possible. We studied with special care the terminal manifestations of the disease. In cases of hypertension with renal involvement "uremia" is often reported as a cause of death. Regarding uremia as a complex syndrome with renal, cerebral and cardiac elements we feel that the term as such is misleading and, if used, requires strict definition. This is especially important in "malignant" hypertension, and we shall elaborate the point when discussing these cases.

### *Analysis of Clinical and Histological Data*

Our material was drawn from consecutive clinical and autopsy records for 1932, 1933 and 1934.\* All cases showing evidence of hypertension were investigated. From these, diffuse glomerulonephritis, ascending contraction and other obviously renal conditions were eliminated by the methods discussed above. The remaining series, in all 250 cases, were provisionally regarded as vascular or

\* From the Mallory Institute and Second and Fourth Medical Services of the Boston City Hospital.

"essential" hypertension. There was in these cases no evidence of antecedent renal disease. A provisional separation of this series into benign and malignant groups was made on the basis of Fahr's criteria, *i.e.* productive endarteritis and necrotizing arteriolitis in the kidney. Borderline cases showing these arterial changes in atypical form or distribution were placed in a separate class, the significance of which is discussed under the malignant group. For reasons which will become obvious later, we have used the clinical expressions benign and malignant hypertension, rather than the morphological term "nephrosclerosis."

### *A. Benign Hypertension*

A careful analysis of the degree of renal involvement in these cases was first made from the clinical and histological aspects in an attempt to substantiate or disprove Fahr's conception of a true renal decompensation in benign hypertension. On the basis of this analysis the cases fall into four groups. The findings are summarized in Table I.

(1) *Benign Hypertension with No Renal Involvement:* This is the largest group, constituting some 60 per cent of the whole. Clinically, the concentrating power of the kidneys is within normal limits, and there is no elevation of non-protein nitrogen of the blood. Histologically the kidneys of this group show no tubular dilatation and no glomerulitis. Arteriosclerosis is present in all degrees with an average comparative figure of  $4\frac{1}{2}$ .

(2) *Benign Hypertension with "Extrarenal" Nitrogen Retention:* (15 per cent of cases.) Elevation of non-protein nitrogen is in these cases attributable to diminished blood flow through the kidney arising from cardiac failure or some other extrarenal cause. Concentrating power is unimpaired, however, and histologically there is no tubular dilatation and no glomerulitis. The degree of arteriosclerosis is essentially the same as in Group 1.

(3) *Benign Hypertension with Renal Impairment:* (10 per cent of cases.) These cases show the early stages of renal involvement. The non-protein nitrogen of the blood is within normal limits but concentration-dilution tests show slight to moderate impairment of function, the maximum specific gravity being 1021. Histologically we find slight to moderate tubular dilatation but no glomerulitis. Ar-

teriosclerosis is more severe than in Groups 1 and 2 with a comparative figure of  $6\frac{1}{2}$ .

(4) *Benign Hypertension with Renal Decompensation*: This group constitutes 11 per cent of the cases. The main histological features are focal alterative glomerulitis of the types already described, considerable tubular dilatation and severe arteriosclerosis. Clinically the concentrating power of the kidneys is impaired, specific gravities of urine in the Volhard test falling between 1012 and 1017. The

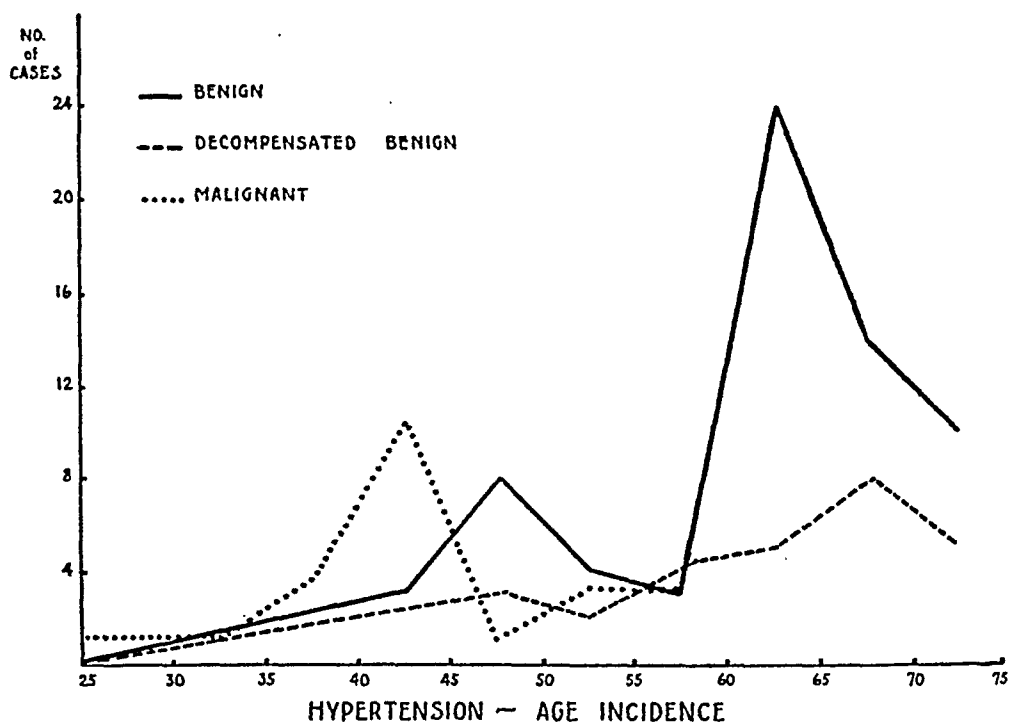
TABLE I

*Renal Involvement in Benign Hypertension and Kidney Changes*

Groups	Per-centage	Average specific gravity of urine	Limits of N. P. N.	Average arterio-sclerosis	Tubular dilatation	Glomeru-litis
1. No renal involvement	60	Within normal limits	Within normal limits	$4\frac{1}{2}$	Negative	Negative
2. Extrarenal nitrogen retention	15	Within normal limits	50-135	4	Negative	Negative
3. Renal impairment	10	1012-1021	33-60	$6\frac{1}{2}$	Slight to moderate	Negative
4. Renal decompensation	11	1012-1017	57-200	8	Moderate to considerable	Present

non-protein nitrogen of the blood is elevated in all cases except two, the limits being 57-200 with an average value of 73 mg. per 100 cc. It is necessary to review the evidence which convinced us that this is a true renal decompensation occurring in the course of benign hypertension. In the first place the clinical picture up to the stage of renal failure is that of other cases of benign hypertension (Group 1). Uremia plays a subordinate part in the terminal picture, the common causes of death being cardiac failure, coronary thrombosis, cerebral accidents or bronchopneumonia. In a few cases, however, true uremic coma was undoubtedly present. The most characteristic clinical feature is the age group into which these cases fall. When the age incidence is compared with that of Group 1 it is seen that the peak falls several years later (Text-Fig. 1). Histologically the relation of these cases to the previous groups of benign hypertension is even

more clearly brought out. As we pass from Group 1 through the stage of renal impairment to that of insufficiency there is a striking increase in the severity of arteriosclerosis. As seen in the summarizing table (Table I), Groups 1 and 2 show the same average degree of arteriosclerosis ( $4-4\frac{1}{2}$ ), Group 3 an intermediate figure ( $6\frac{1}{2}$ ) and Group 4 a still severer degree (8). This gradation in kidney involvement receives strong support from the existence of Group 3, in which tubular dilatation and impaired concentrating power are alone pres-



TEXT-FIG. 1

ent. When the average kidney weights are similarly compared, the decompensated group shows a greater shrinkage than the cases with no clinical or histological evidence of renal involvement (256 and 326 gm. respectively). We are led to the conclusion, therefore, that if the patient lives long enough the arteriosclerotic process leads eventually to diminution in kidney parenchyma below the limits of its functional reserve. The high percentage of cases with renal decompensation in our series is probably attributable to the relatively late age incidence of our benign hypertensive groups as a whole as is seen from Text-Figure 1. The evidence presented above shows clearly that we are dealing with true renal decompensation as Fahr maintains, and not merely with a nitrogen retention due to terminal



heart failure. The existence of Group 2 with no histological evidence of renal impairment but with extrarenal nitrogen retention emphasizes this point. Lastly, these cases cannot be regarded as malignant hypertension. They fall into a much later age group, they lack the fulminating element of malignant hypertension, hypertensive retinopathy is never observed, and histologically the "specific" arterial lesions of the latter are absent. To Fahr's distinction between essential hypertension and benign nephrosclerosis the above analysis lends no support but rather emphasizes the unity of the whole group of benign hypertension as a primary generalized vascular disease.

### *B. Malignant Hypertension*

We have now to consider those cases which were separated from the main group on account of "specific" arterial lesions — necrotizing arteriolitis or endarteritis. The classical picture of malignant hypertension presented by Volhard and Fahr is that of unusually high blood pressure occurring in relatively young subjects and having a rapid termination in uremia. Hypertensive retinopathy (edema of the disc and retina, with retinal exudates)\* is a constant feature. Histologically necrotizing arteriolitis is found in the kidney and in other organs, usually associated with productive endarteritis. Focal glomerulitis is invariably present. Twelve of our cases fall into the above group. We have, however, made a careful study of the borderline cases previously referred to in which there are discrepancies from the classical picture described above. These cases have led us to the conclusion that the malignant nephrosclerosis of Volhard and Fahr does not give a true picture of the disease "malignant hypertension" but represents only the renal end-stage. Cases exist in which malignant hypertension is present but death occurs before this renal end-stage is reached.

As in benign hypertension, the malignant group can be arranged on clinical and histological grounds in increasing order of renal involvement. Such an arrangement has been made in Tables II and III. Owing to the fulminating nature of the disease, cases with *no renal involvement* are very rare. In the first group of cases, however, (Table II) renal failure plays little or no part in the final picture. Clinically the diagnosis of malignant hypertension is supported by the relatively young age incidence, the unusually high blood pres-

\* The term hypertensive retinopathy is used in this sense throughout the paper.

sure, the presence of hypertensive retinopathy, and the fulminating termination. In certain cases the actual cause of death was obscure, being termed clinically "hypertensive encephalopathy" (*vide infra*) and at autopsy no organic lesion could be demonstrated except in one case which showed multiple small hemorrhages in the cerebral cortex.

Referring again to Table II, there was little or no elevation of non-protein nitrogen of the blood in these cases. Renal impairment, as indicated by diminished concentrating power of the urine, was slight or absent.

The small extent of clinical involvement of the kidney is paralleled in the histological picture. Tubular dilatation is either absent or slight compared with Group II. Glomerulitis is absent in Case 1, and only one instance was found in several sections in Case 4. The remaining members of the group show scanty focal glomerulitis compared with the more extensive changes in Group II. The same holds true for necrotizing arteriolitis. This lesion is absent in Cases 4 and 8. In Case 2 it is confined to the pancreas and adrenal and in the remainder its distribution is so scanty that only occasional examples are seen in going through several sections. Productive endarteritis is found as a diffuse lesion in only two cases and is absent or focally distributed in the remainder.

Finally, Cases 9 to 19 (Table III) show the *fully developed picture with uremia*. Histologically the findings are characteristic and it is to be noted that in this group necrotizing arteriolitis, endarteritis and focal glomerulitis are as a rule far more severe in extent than in the previous cases.

The separation of the above cases into groups is somewhat artificial. They show a gradual increase in kidney involvement. The arrangement is made solely to bring out the relation of malignant hypertension as such to the end-stage which has been termed malignant nephrosclerosis. According to Fahr's criteria cases of the first group showing no necrotizing arteriolitis cannot be regarded as malignant nephrosclerosis, but we feel that their clinical course warrants the diagnosis of malignant hypertension — a malignant hypertension which proves fatal from extrarenal causes before the kidneys are seriously involved.

As we pass from Group I to Group II, the extensiveness of the lesions in the kidney increases and the individual elements, which at

TABLE II

*Malignant Hypertension. Group I*

Case No.	Autopsy No.	Age	Blood pressure	N. P. N.	Specific gravity	Retinopathy	Glomerulitis	Productive Endarteritis		Necrotizing Arteriolitis		Tubular dilatation	Cause of death
								Kidney	Other organs	Kidney	Other organs		
1	31-68 Ch.-H	yrs. 12	mm. Hg. 260/160 200/150	Nor- mal	1005-1020	+	Negative	Diffuse	Present	Very sparsely	Negative	Negative	Septicemia
2	32-231	45	220/130	24	1004	+	Occasional	Negative	Negative	Negative	Present in pancreas and adrenal	+	"Encephalopathy"
3	32-341	52	245/120 270/130	45-29 42-43	1010-1022	+	Occasional	Focal slight	Present in pancreas and adrenal	Negative	Negative	++	Bronchopneumonia, cerebral softening
4	33-10	24	220/140 245/140	35-56 41	1006-1022	+	Only 1 found	Moderate	Negative	Negative	Negative	++	"Encephalopathy"
5	33-264	45	260/150 240/150	30-34	1008-1022	+	Very few	Slight in some small arteries	Negative	Negative (occasional fibrinoid degeneration)	Adrenal (fibrinoid degeneration)	+	"Encephalopathy"
6	33-714	40	230/150 180/110	33-66	1010-1014	+	Occasional	Some, focal slow in type	Negative	Occasional	Occasional (fibrinoid degeneration)	++	Multiple punctate hemorrhages in brain
7	34-U20	36	260/140	23-50	1003-1009	+	Occasional	Diffuse	Adrenal severe	Very sparsely	Negative	+++	Adrenalectomy 3 weeks ago. Pulmonary embolism
8	34-84	41	250/150	33	1008-1012	+	Occasional	Some, focal slow in type	Pancreas adrenal fairly diffuse	Negative	Negative	++	Bronchopneumonia

TABLE III

*Malignant Hypertension. Group II*

Case No.	Autopsy No.	Age	Blood pressure	N. P. N.	Specific gravity	Retinopathy	Glomerulitis	Productive Endarteritis		Necrotizing Arteriolitis		Tubular dilatation	Cause of death
								Kidney	Other organs	Kidney	Other organs		
9	32-88	3rs. 42	mm. Hg. 220/110	80 248	1007-1010	+	Occasional	Diffuse	Present in pancreas	Occasional	Present in pancreas	++	Uremia
10	32-271	29	260/150	33-35 21-120	1005-1018	+	Frequent	Occasional		Extensive		++	Uremia
11	32-533	45	235/120	82-190 265	1010-1016	+	Occasional	Diffuse	Present in pancreas	Negative	Negative	++	Uremia
12	33-64	41	260/150	205 190	1010-1012	+	Frequent	Considerable	Negative	Fairly diffuse	Negative	++	Uremia
13	33-327	45	200/120 190/120	175 165		+	Frequent	Diffuse	Present	Frequent	Present in pancreas	++	Uremia
14	33-440	43	215/155	45-67 53	1004-1009	+	Occasional	Occasional	Present in pancreas	Extensive	Negative	++	Bronchopneumonia
15	33-514	46	250/140	?	1005-1016	+	Frequent	Diffuse	Present in pancreas	Extensive	Negative	++	Encephalopathy? Uremia?
16	33-722	52	230/130	175	1015-1018	+	Occasional	Diffuse	Negative	Occasional	Negative	++	Uremia
17	33-727	40	240/170 260/170	35-41 86-207	1006-1015	+	Occasional	Diffuse	Present	Extensive	Present only in cecum	++	Uremia
18	34-388	41	200/120	150	1012	+	Frequent	Not diffuse	Negative	Extensive	Negative	++	Uremia
19	S-34-3225	46	220/130	133	?	+	Occasional	Fairly extensive	?	Fairly extensive		++	Uremia

first are scanty and irregular in distribution, combine more and more to form the histological entity or "full blown picture" of malignant nephrosclerosis. Thus, the histological diagnosis of this condition does not depend on any single specific lesion but can be made only from a consideration of the whole histological picture.

Concerning the nature of the nitrogen retention in malignant nephrosclerosis, the same arguments which were employed in benign hypertension with renal decompensation apply to the malignant type. Impaired concentrating power and tubular dilatation point to a true renal origin so that cardiac failure is only secondarily responsible, if at all, for elevation of the non-protein nitrogen of the blood.

So far we have regarded benign and malignant hypertension as two different processes, the former incident in relatively old individuals associated with varying degrees of arteriosclerosis and in some cases gradually leading to moderate renal insufficiency, the latter occurring in younger subjects and having a fulminating termination usually involving the kidney, which shows characteristic arterial lesions. The difference may be considered as one in the reaction of the arteries of the individual to the vasospastic process, young subjects being more "reactive" than older individuals.

There is a second group of *borderline or non-classical cases* which supports this idea and also might answer the question whether "benign hypertension ever becomes malignant." These cases (Table IV) represent a transition between the decompensated benign group and the malignant nephrosclerosis. Compared with the latter, they belong to a relatively later age period and have a less fulminant progress. One case showed hypertensive retinopathy characteristic of the malignant hypertension. Renal impairment is present with moderate nitrogen retention, but the "uremic process" as such plays a less conspicuous part in the terminal picture — recalling in this respect the cases in Group I. Endarteritis is found associated with arteriosclerosis and is often of the transitional type which has been described. Necrotizing arteriolitis, however, is only slight in extent being frequently associated with the change we have termed "fibrinoid degeneration." We feel that these cases represent a malignant type of hypertension incident in relatively old individuals, often superimposed on long-standing benign hypertension, and in this sense corresponding to the "Umschlag" of Volhard.

TABLE IV

*Malignant Hypertension. Group III. (Transitional Group)*

Autopsy No.	Age	Blood pressure	N. P. N.	Specific gravity	Retinopathy	Glomerulitis	Productive endarteritis		Necrotizing arteriolitis		Tubular dilatation	Cause of death
							Kidney	Other organs	Kidney	Other organs		
32-553	yrs. 62	mm. Hg. 220/140	95	1008	Not examined	Occasional	Some focal slow type		Very sparsely		+++	Uremia
34-406	55	180/100	67	1014-1020	Not examined	Occasional	Negative	Negative	Occasional	Present in pancreas	+++	Cardiac failure
34-612	62	180/100	58	1004-1010	Not examined	Occasional	Negative	Negative	Occasional	Negative	++	Cardiac failure
34-707	60	260/135	52-97	1012-1018	+	Occasional	Diffuse	Present in pancreas	Negative	Negative	+++	Uremia

The summarizing classification of benign and malignant hypertension may therefore be outlined as follows:

A. *Benign Hypertension*

- |   |   |     |
|---|---|-----|
| (1) No renal involvement  | } | 75% |
| (2) Extrarenal nitrogen retention                               |   |     |
| (3) Renal impairment  |   | 10% |
| (4) Renal decompensation (decompensated benign nephrosclerosis) |   | 11% |

*Intermediate or Transitional cases* ("Umschlag" of Volhard) less than 1%

B. *Malignant Hypertension*

- |  |   |     |
|--|---|-----|
| (1) No renal involvement   | } | 4%* |
| (2) Renal impairment   |   |     |
| (3) Renal decompensation — the "malignant nephrosclerosis" of Volhard and Fahr |   |     |

## DISCUSSION

### I. *Benign Hypertension*

#### A. *Hypertension and Renal Arteriosclerosis*

(1) *Special Significance of Arteriolar Changes:* Our observations confirm the work of other observers on the relation of arteriosclerosis to hypertension. Although a definite parallelism exists between the two, it is in many instances imperfect. Certain authors regard arteriolar sclerosis as "morphologically characteristic" of hypertension (Herxheimer and Schulz,<sup>9</sup> Fahr,<sup>1</sup> Bell and Clawson<sup>5</sup>), but it appears from our data that the relationship is equally true for the medium sized vessels. Arteriosclerotic changes in some cases predominate in the arterioles, in others in the interlobular vessels. Involvement of the arterioles alone is decidedly rare. In view of the variations in statistics one should regard with caution any attempt to attribute a diagnostic significance to arteriolar changes.

(2) *Hypertension as the Cause of Renal Arteriosclerosis:* Several factors prevent us from accepting such a statement unreservedly.

\* In collecting cases of malignant hypertension, the records of all services of the Boston City Hospital were drawn upon. The percentage incidence is, however, based only on those cases from the 2nd and 4th Medical Services.

Cases of renal arteriolosclerosis without hypertension, although rare, have been described (Löhlein,<sup>10</sup> von Monakoff,<sup>11</sup> Fahr,<sup>1</sup> Kimmelstiel<sup>8</sup>). We also encountered a few examples of this type. Long-standing experimental hypertension may be produced in animals by section of the aortic nerves or by kaolin injections into the cisterna magna, but apparently arteriosclerosis does not follow such hypertension (Hamperl and Heller,<sup>12</sup> Nordmann<sup>13</sup>). Graybiel, Allen and White<sup>14</sup> have performed muscle biopsies on the upper and lower extremities of patients with coarctation of the aorta. They failed to find any significant difference in the arterioles although there is in such cases a much higher blood pressure in the arm than in the leg. Fahr has recently pointed out that in cases of severe renal arteriolosclerosis, these changes are absent from the vessels of the kidney capsule. Since both groups of vessels originate from the same artery and are subject to the same blood pressure, it appears that hypertension *per se* cannot be regarded as the cause of the arteriosclerosis. Fahr interprets this observation as supporting his theory of the renal origin of the hypertension. Such a distribution of the arterial lesions can, however, be equally well explained on the assumption that arterial strain is proportional to functional activity in the area of supply.

The conclusion is that hypertension may be considered as an accelerating factor in the development of arteriosclerosis as Aschoff maintains and Fahr to some extent admits, but the reaction of different vascular regions to the hypertension is not uniform and is determined in some way by the functional activity of the area supplied.

(3) *Renal Arteriosclerosis as a Cause of Hypertension:* We have already referred to cases of hypertension in which renal arteriolosclerosis is absent. There is considerable difference of opinion as to the frequency of these cases. According to Herxheimer and Schulz,<sup>9</sup> renal arteriolosclerosis is found in 97 per cent of cases of cardiac hypertrophy with hypertension. Bell and Clawson<sup>5</sup> place the figure at 90 per cent. Fishberg<sup>15</sup> states that intact kidneys are decidedly uncommon. Such numerical values are based on a purely quantitative estimation which is open to subjective errors. If mechanical obstruction to the circulation through the kidney is to be considered as a contributory factor to the hypertension, it has to be borne in mind that less than 50 per cent of cases show a completely diffuse arteriolosclerosis. Jaffé<sup>16</sup> believed that dilatation of the vas afferens,



which he frequently observed in association with very early degenerative changes in the glomerulus, pointed to a primary circulatory disturbance which reflexly produced the hypertension. This finding is, however, inconstant; hence the evidence cannot be regarded as conclusive. On the positive side animal experiments undoubtedly indicate that obstruction to the kidney circulation produces hypertension (Goldblatt *et al.*<sup>17</sup>). In human kidneys Kimmelstiel has shown by postmortem perfusion that in certain cases of benign hypertension an actual obstruction to the circulation exists. These cases are of the type we have described above as decompensated benign nephrosclerosis. In this group, therefore, where impairment of renal function is present, one may be justified in regarding the arteriosclerotic process in the kidneys as a factor in augmenting or maintaining the hypertension. In all other cases we must regard hypertension and arteriosclerosis as undergoing a parallel development as age advances.

(4) *Functional Disturbances in Arteriosclerotic Kidneys (Renal De-compensation)*: This group has been recognized by various observers but different interpretations have been placed upon it. Schürmann and MacMahon<sup>18</sup> consider the cases as transitional to malignant nephrosclerosis. Murphy and Grill<sup>19</sup> similarly maintain that no clear distinction exists between the two groups. Under Volhard's influence most authors incline to the opinion that when renal failure occurs in benign hypertension we should consider the disease to have entered on the malignant phase or "Umschlag" (Volhard,<sup>3</sup> Lange,<sup>20</sup> Fishberg<sup>15</sup>). Exception is made to this interpretation in cases where nitrogen retention is attributable to cardiac failure and disappears as the cardiac condition improves. We hope the analysis of our data clarifies the situation by establishing the decompensated benign group as a distinct entity, and by bringing out its true relation to malignant hypertension. We have pointed out the clinical and histological features which differentiate the two conditions and have contrasted these cases with those of extrarenal nitrogen retention. There is no doubt that cardiac failure may be present and may be the precipitating cause of renal failure, but the primary and most important element is the condition of the kidney itself. Although this is admitted by some writers as a possibility (Volhard,<sup>3</sup> Lichtwitz,<sup>4</sup> Fishberg,<sup>15</sup> Lange<sup>20</sup>), the frequency with which it occurs is not appreciated.

The significance and pathogenesis of the glomerular lesions in decompensated benign nephrosclerosis remain to be discussed. Are these to be regarded as the cause or the effect of renal insufficiency, or are the two manifestations attributable to a common cause? The first possibility appears extremely unlikely on account of the focal nature of the glomerulitis and the very scanty distribution of the lesion in many cases. Considering the second possibility it is obvious that nitrogen retention as such does not produce alterative glomerulitis, as the control Group 2 with extrarenal nitrogen retention clearly demonstrates. Moreover, there exist occasional cases of decompensated benign nephrosclerosis with no evidence of elevated non-protein nitrogen. Functional impairment (as indicated by diminished concentrating power and tubular dilatation) is, however, invariable and in view of the overwhelming frequency with which nitrogen retention is present in cases with glomerulitis we cannot exclude renal impairment as an etiological factor. A consideration of the third possibility — that renal insufficiency and alterative glomerulitis are attributable to the same cause — may throw further light on the matter. Can ischemia be regarded as the common cause? The common arteriosclerotic lesion of the glomeruli, produced by slow arterial occlusion, is an atrophic degenerative change. If we postulate a sudden ischemic process or spasm as the cause of the acute necrosis observed in alterative glomerulitis, a corresponding change should be discernible in the tubules. In fact, the tubular apparatus is more susceptible to vascular damage than the glomeruli. Such changes are not found. At most we encounter hyaline droplet degeneration in the tubules. Russell<sup>21</sup> pointed out that glomerulitis occurs in the vicinity of acute renal infarcts. Klemperer and Otani<sup>22</sup> also reported necrotizing arteriolitis in the same location, and regard the finding as suggestive evidence of the ischemic origin of these changes. We have examined a series of renal infarcts and have identified examples of the alterative glomerulitis already described. The corresponding tubules, however, show no acute necrosis but rather a necrobiotic process with regeneration, an observation which prevents us from ascribing the glomerular or arteriolar lesions to pure ischemia. The occurrence of glomerulitis and arteriolitis in the vicinity of acute renal infarcts suggests, indeed, a more probable explanation of their pathogenesis. We cannot escape the conclusion that in this very situation diffusible toxins are produced by the breakdown

of kidney tissue. Toxic and ischemic factors combined may produce alterative glomerulitis. The possibility, therefore, remains that in decompensated benign nephrosclerosis retained products may act as toxins on glomeruli which are already damaged by severe arteriosclerosis. Cases with no nitrogen retention are difficult to explain on this basis but we might suggest that toxic substances may be retained before the non-protein nitrogen rises appreciably, or that, owing to the fluctuant nature of the latter, it may be normal at the time of the determination. In view of the inconclusive character of the evidence, however, we still entertain the possibility of an extrarenal toxin which may produce vascular spasm with coincident renal failure and alterative glomerulitis.

## *II. Malignant Hypertension and Malignant Nephrosclerosis*

In the presentation of cases of malignant hypertension we have briefly outlined our conception of the relation of malignant hypertension to malignant nephrosclerosis and have pointed out that borderline cases which are difficult to explain on the basis of Fahr's classification are in reality essential to an understanding of the true nature of the disease. We must now consider the new interpretation to be placed on the specific vascular lesions in relation to the clinical picture, both from the diagnostic point of view and also with reference to the pathogenesis of the condition.

In the first place we have attempted to readjust the emphasis which hitherto has been laid on specific arterial lesions in the kidney and transfer it to the clinical picture of malignant hypertension. The clinical features, which in our opinion characterize this type of hypertension, are the relatively young age incidence, the unusually high blood pressure, the occurrence of hypertensive retinopathy and the fulminating progress of the disease, with cerebral manifestations, designated, for want of greater knowledge, "hypertensive encephalopathy." Such manifestations include a variety of symptoms, among which may be mentioned severe headaches and dizziness, disorientation and loss of memory, transient paresis and paresthesias, occasionally convulsions, visual disturbances and finally coma, which may occur in the absence of gross cerebral lesions or nitrogen retention. The presence of such a clinical picture, which has been called "pseudo-uremia" by Volhard, distinguishes these cases from the benign type of hypertension. The most important inference result-

ing from our investigation is that malignant hypertension as such precedes the stage of renal involvement — the proof being based on histological as well as clinical evidence. It appears that too much emphasis has hitherto been placed on the renal end-stage — malignant nephrosclerosis. The rigid histological criteria introduced by Fahr must therefore be relaxed and extended to embrace cases which on clinical grounds should be included in the group of malignant hypertension.

### *The Specific Arterial Lesions*

We have already stated that the cases described under malignant hypertension were primarily separated on the basis of "specific" arterial lesions — productive endarteritis or necrotizing arteriolitis. The distribution of these lesions throughout the group, however, is not consistent with the significance attributed to them by Volhard and Fahr. We shall now attempt to elaborate a fuller conception of the nature of these changes.

(1) *Productive Endarteritis*: Diffuse endarteritis in the small or medium sized arteries of the kidney is an unmistakable diagnostic criterion of malignant hypertension. In several of our cases, however, it is either entirely absent or inconspicuous, so that it cannot be regarded as an invariable feature of the disease. We agree with Klemperer and Otani<sup>22</sup> that productive endarteritis should be regarded as an accelerated form of arteriosclerosis. The transitional type of lesion we have already described supports this view and makes it difficult to regard the change as inflammatory (Evans<sup>23</sup> and Fahr<sup>1</sup>). Productive endarteritis differs from the purely degenerative arteriosclerosis in one important respect however, namely, that in the diffuse form it is invariably associated with high blood pressure. We may, therefore, justifiably regard it as a response to a particularly severe type of hypertension. The rate of development of the endarteritic process may be extremely rapid. Weiss, Parker and Robb<sup>24</sup> described a case of malignant nephrosclerosis in which one kidney was removed at operation 67 days before death. Through their courtesy we have been able to examine sections of both kidneys and observed a striking increase in the extent and severity of productive endarteritis during this interval. We may state, therefore, that productive endarteritis may be absent in the early stages of malignant hypertension, develops rapidly during the course of the

disease, is present as a diffuse lesion in the kidney and other organs in fully developed cases, and in the diffuse form is never seen in the absence of hypertension. We cannot, however, explain the lesion simply as a reaction to a severe type of vascular spasm. Prinzmetal and Wilson<sup>25</sup> have shown that the vasospastic process in malignant hypertension is universally distributed. The incidence of endarteritis is, however, by no means universal — usually being confined to the organs of the splanchnic area. We therefore make the assumption that the vessels in certain regions are more susceptible to the vasospastic process, a susceptibility which may be in some way related, as we have already suggested in discussing arteriosclerosis, to the functional activity of the area of supply. This view is consistent with the observation made by Fahr that endarteritis and arteriolitis are not found in the vessels in the kidney capsule. It is necessary, however, to mention the paper by Kernohan, Anderson and Keith<sup>26</sup> who describe thickening of the arterial wall in the voluntary muscles in hypertension. It is surprising that similar investigations (Graybiel *et al.*<sup>14</sup>) in cases of coarctation of the aorta did not reveal any difference in the arterioles in the arm and leg. In order to explain this discrepancy we may point to the recent experiments one of us<sup>25</sup> has made in which it was shown that the mechanism of arteriolar constriction in generalized hypertension is apparently different from that in coarctation of the aorta. The former has to be interpreted as intrinsic vascular spasm whereas the latter has been shown to be vasomotor in origin. Graybiel, Allen and White<sup>14</sup> suggest a similar explanation although experimental support was not available at that time.

(2) *Necrotizing Arteriолitis*: Since necrotizing arteriolitis in the kidney may be slight or even absent in cases with malignant hypertension we cannot justifiably regard it as causing the hypertension. Arteriолitis differs from productive endarteritis in its relation to renal failure. Cases which show a fulminating uremic termination with gross nitrogen retention (Group II), in general show severe necrotizing arteriolitis. In the pre-renal stage, however (Group I), this feature is slight or absent. The extent and severity of the focal glomerulitis also show a parallel relationship with the renal function. The pathogenesis of the arteriolar necrosis presents a similar problem to that of the origin of alterative glomerulitis in decompensated benign nephrosclerosis. The inconstancy of the lesion prevents us from re-

garding it as the cause of renal insufficiency. Its occurrence with alterative glomerulitis in the vicinity of acute infarcts suggests the association of toxic and acute ischemic factors in its production. We have referred above to evidence of vascular spasm in malignant hypertension. Such spasm has been observed in the retinal vessels and its existence is indicated on the pathological side by cases of cortical necrosis of the kidney and by the occurrence of "Fleckmilz" and "Fleckpancreas" in malignant nephrosclerosis.

That spasm alone does not cause arteriolitis is evident from the absence of this lesion in Raynaud's disease and acute eclampsia where vascular spasm is undoubtedly present. Klemperer and Otani<sup>22</sup> suggest that endarteritis in larger radicles may produce ischemic damage in the corresponding arterioles, thereby precipitating arteriolar necrosis. Since, however, necrotizing arteriolitis occurs in the absence of endarteritis (see also our Case 2), these authors were led to subdivide malignant nephrosclerosis into two types: one in which arteriolar necrosis is assumed to follow endarteritis ("accelerated arteriosclerosis"), the other in which true necrotizing arteriolitis is regarded as the primary lesion. Schürmann and MacMahon similarly attempted to distinguish two forms of malignant nephrosclerosis — an exogenous and an endogenous form — on the basis of a difference in distribution of the arteriolar lesion. We feel that the evidence is insufficient to justify such distinctions. In the first place the separation of arteriolar necrosis and necrotizing arteriolitis is unjustifiable since transitions frequently occur from one to the other. Secondly there is no constant relation between endarteritis and arteriolar necrosis or arteriolitis. The distribution of the lesions in the arterioles is indeed very irregular and we feel cannot be regarded as an adequate basis for subdivision of cases. Such variations as occur are attributable to the different stages of development of the disease rather than to differences in pathogenesis. It is impossible to escape the fact that in malignant hypertension the development of both arteriolitis and glomerulitis is closely related to renal insufficiency. By analogy with decompensated benign nephrosclerosis it seems reasonable to regard the renal failure as the direct result of acute functional ischemia. Whether some toxin, producing the vascular spasm, produces also the glomerulitis and arteriolitis, or whether, alternatively, the combination of acute ischemia and retained toxic substances be regarded as the cause remains an open

question. Whatever their pathogenesis, these manifestations must be considered as essentially characteristic of the acute renal end-stage of malignant hypertension, independent of the endarteritis and in certain cases appearing before the latter has developed. When death occurs before this end-stage is reached (Group I) necrotizing arteriolitis may be entirely absent.

### *Malignant Hypertension*

*Etiology:* We have been led to postulate both a toxic and a spastic factor to explain the characteristic lesions of malignant hypertension. The only exogenous toxin of known etiological significance is lead. Recent work has shown, however, that other conditions may act as precursors of malignant hypertension and lead ultimately to malignant nephrosclerosis. Such are basophil adenoma of the pituitary<sup>27</sup> and eclamptic toxemia of pregnancy.<sup>28</sup> This suggests that any hypertensive state may give rise to the malignant form of hypertension. There is considerable evidence that acute glomerulonephritis, the most common cause of hypertension in young subjects, may similarly act as a precursor of malignant hypertension. Persistent hypertension has been reported to follow acute glomerulonephritis even though the renal lesion has apparently healed (Longcope,<sup>29</sup> Van Slyke<sup>30</sup>). It might be expected that hypertension of such an origin could pass over to the malignant form. Volhard<sup>3</sup> has reported a case of glomerulonephritis which suggests this sequence of events (case Joh. Ei., p. 1439). The histological diagnosis of malignant nephrosclerosis was made, although from the description it is not clear whether terminally the glomerular lesion was focal or diffuse. The following case is an example of acute nephritis in which apparently the diffuse glomerular lesion healed, and which was followed later by malignant nephrosclerosis.

CASE 1. E. S., first admitted May, 1924, aged 8 years, with 2 days history of bloody urine followed by headaches and convulsions coming on after an attack of acute tonsillitis. *Urine:* Specific gravity 1010 to 1015; albumin, slight trace.

Well until second admission August, 1933. Three months pregnant.\* Blood pressure 228/158. *Urine:* Specific gravity 1006; large trace of albumin; occasional white and red blood cells in sediment. Pregnancy terminated.

Third admission December, 1934. Two and a half months pregnant. Blood

\* In view of the early stage of pregnancy it is unlikely that a hypertension of such severity could be attributable to a pregnancy toxemia.

pressure 230/100. *Urine:* Specific gravity 1009; large trace of albumin. Therapeutic abortion and sterilization performed.

Final admission February, 1935, complaining of increasing dyspnea and abdominal swelling. *Physical Examination:* Orthopneic, pale, signs of heart failure. Fundi not well seen. Blood pressure 270/165. *Urine:* Specific gravity 1008; large trace of albumin; granular casts and occasional red cells in sediment. Non-protein nitrogen 150 mg. per cent rising to 325. Developed pericardial friction rub and died in uremic coma.

### *Histological Findings in the Kidney*

Multiple areas of fresh anemic infarction with a broad hemorrhagic border in the cortex.

*Arteries:* Severe diffuse endarteritis of large, medium and small vessels. Extensive necrotizing arteriolitis. Fibrin thrombi are present in necrotic vessels within the substance of the infarcts but are not seen outside these areas.

*Glomeruli:* Majority are normal. Remainder show increase in nuclei, necrosis of capillary walls, occasional adhesions, a few polymorphonuclear leukocytes present.

*Tubules:* Considerable tubular hyperplasia.

The conception that acute glomerulonephritis may act as a precursor of malignant hypertension is consistent with the occurrence of arteriolitis and endarteritis in the kidney in chronic glomerulonephritis. The high blood pressure which develops in the later stages of diffuse glomerulonephritis is usually moderate in degree, but cases are not infrequently encountered, especially in young subjects where the blood pressure is extremely high. In such cases hypertensive retinopathy is frequently present and the rapid downhill course of the disease is more characteristic of malignant hypertension than of chronic glomerulitis. The following is a typical example.

CASE 2. J. P., male, aged 27 years, admitted Nov. 8, 1932, with a 2 years history of "kidney trouble." Frequency day and night and hematuria. There was a history of severe headache, shortness of breath, swelling of face and misty vision for 1 week following a cold in the head.

*Physical Examination:* This revealed a pale, ill-looking man, orthopneic and coughing up blood-stained sputum. Retinal examination showed papilloedema with extensive exudates and hemorrhages. Blood pressure 240/140 mm. Hg.

*Urine:* Specific gravity maximum 1012; large trace of albumin; many red cells and leukocytes in sediment.

*Blood:* Non-protein nitrogen 130 mg. per 100 cc.

Patient died in uremia on day after admission.

*Macroscopic Examination of Kidneys:* Combined weight 160 gm. Capsule strips with difficulty from yellowish gray, coarsely granular surface. Cut surface boggy. Cortex diminished in size.

*Histological Examination of Kidneys:* Chronic diffuse glomerulonephritis. Every glomerulus involved. Fairly extensive necrotizing arteriolitis and productive endarteritis affecting small vessels only.



The difficulty in making a histological diagnosis in such cases arises from the simultaneous occurrence of diffuse glomerulonephritis and the arterial lesions — endarteritis and necrotizing arteriolitis. Two theories have been suggested:

(1) The arterial lesions are regarded as secondary to the glomerular inflammation. This explanation, which is maintained by Fahr, is apparently supported by the fact that the vascular lesions are confined to the kidney. If, however, the arteriolitis and endarteritis are regarded as part of a generalized vascular disease their occurrence only in this situation can be explained on the basis of the excessive vascular strain in an already damaged kidney. In pure malignant nephrosclerosis the vascular lesions may similarly be found only in the kidney. Hence the fact that arterial and arteriolar changes in glomerulonephritis are not found outside the kidney does not disprove the theory that they depend on a generalized vascular disturbance.

(2) There remains the possibility that we are dealing with coincident malignant nephrosclerosis and chronic glomerulonephritis. The frequency of this association, however, suggests a closer relation between the two conditions. It has frequently been stated that arteriolitis also occurs in acute nephritis (Löhlein,<sup>31</sup> Fishberg<sup>32</sup> and others) and the explanation has been offered that the same toxin produces both the glomerulonephritis and the arteriolitis. Such a toxin may be allergic in origin (Masugi<sup>33</sup>). It appears moreover that acute nephritis is not to be regarded as a purely renal disorder but rather as a general vascular disturbance which may manifest itself before the kidney shows signs of involvement. Assuming that one toxin (? allergic) may produce both the arteriolar and glomerular lesions in acute nephritis, the same explanation may hold for the lesions found in chronic glomerulonephritis. The acute stage of the disease may be regarded as resulting in a hypersensitive state of the general arterial system as well as of the glomeruli. In its progress the disease may involve either the glomeruli or the blood vessels, or both. Accordingly we may encounter any of the above mentioned sequelae of acute glomerulonephritis, *i.e.* chronic diffuse glomerulonephritis, malignant nephrosclerosis, or chronic glomerulonephritis with vascular lesions in the kidney.

*Periarteritis Nodosa and Malignant Nephrosclerosis*

The close similarity between these conditions in many instances led Fahr to the opinion that malignant nephrosclerosis might be regarded as a special form of periarteritis nodosa in which the arteriolar lesions were for the most part confined to the kidney — a view which agreed with his theory of the renal origin of malignant hypertension. If, however, we are correct in regarding malignant hypertension as a primary generalized vascular disturbance, and the renal vascular lesions as secondary, it is necessary to find an explanation for the association of malignant hypertension and periarteritis nodosa, especially since Volhard states that in these cases the hypertension is of the so-called "pale" type. Since the summarizing articles on periarteritis nodosa do not discuss in detail the relation of kidney involvement to hypertension, especially in its malignant form, we have made an analysis of the original case reports in the literature.

Cases with insufficient information concerning blood pressure, heart weight or kidney involvement were eliminated. Where a diagnosis of diffuse glomerulonephritis was made or where kidney involvement was definitely stated to be focal the case was also excluded. There remained some 75 cases with a diffuse distribution of the arterial lesion in the kidney — so-called "infarcted contracted kidneys." Forty-seven cases (62 per cent) showed evidence of hypertension (in 5 cases based on cardiac hypertrophy at autopsy). In 28 cases (37 per cent) a normal blood pressure was observed (in 5 cases no note was made of the blood pressure but the heart weight was normal at autopsy). Eighteen of the cases with hypertension showed a systolic pressure at or above 200 mm. Hg.

*Relation of Hypertension to Kidney Involvement:* We have pointed out that the majority of cases (62 per cent) with diffuse arterial lesions in the kidney showed hypertension. Where diffuse lesions in the kidney were absent no elevation of blood pressure was found. On the clinical side complete renal function tests were only occasionally available, hence the occurrence of red blood cells, albumin and casts in the urine had to be taken as clinical evidence of renal involvement. Eighty-four per cent of cases with hypertension showed such involvement but similar findings were also present in 37 per cent of cases without hypertension. Thus, as has previously

been stated, there appears to be no rigid relation between hypertension and renal involvement in periarteritis nodosa.

*The Nature of the Hypertension:* We usually find that the elevation of blood pressure in periarteritis nodosa is gradual in development, moderate in severity and, in cases where a full description is given, appears to be preceded by the renal involvement. This suggests that we are in fact dealing with a renal hypertension. Cases are, however, observed where extensive arterial lesions are present in the kidney with death in uremia but with no hypertension. It is possible that the diffuse involvement of the heart muscle frequently observed in periarteritis nodosa may in such cases prevent the blood pressure from rising.

An attempt to separate cases of the malignant type was made by paying special attention to the ophthalmoscopic findings. In 15 cases with hypertension where examination of the fundus oculi was reported, 7 were normal, in 6 of these <sup>34-39</sup> at a time when signs of renal insufficiency were already present (*i.e.* diminished concentrating power of the urine or raised non-protein nitrogen). Eight cases showed signs of retinopathy, but whether or not this could in all cases be considered of the malignant type is doubtful. The reports of these cases were, however, analyzed in an attempt to determine whether the hypertension was preceded by renal involvement. We came to the conclusion that such was not invariably the case. In 5 cases <sup>40-44</sup> hypertensive retinopathy and renal insufficiency were already present on the patient's admission to the hospital. In 2 cases, however, there was a history of high blood pressure preceding the periarteritis nodosa by 7 years <sup>45</sup> and 5 years.<sup>46</sup> In only 1 case did retinopathy appear while renal insufficiency was developing.<sup>47</sup> It may be of interest to notice that in 2 cases <sup>48, 49</sup> a previous history of lead poisoning is mentioned.

From the literature, therefore, it appears that the hypertension associated with periarteritis nodosa is only occasionally of the malignant type. More often it is in the nature of renal hypertension — moderate in severity, late in development, and lacking the retinal signs which characterize malignant hypertension. Malignant nephrosclerosis and periarteritis nodosa differ therefore in certain important respects. In the former, hypertension is of primary vascular origin, is malignant in type and terminates in renal failure. Histologically productive endarteritis is the most characteristic

arterial lesion and is regarded as the result of a severe prolonged vascular spasm. Arteriolitis on the other hand appears to be a terminal manifestation and is related more closely to renal insufficiency than to hypertension.

In periarteritis nodosa inflammatory lesions of the vessels occur as the primary event and, as in malignant nephrosclerosis, appear to be more closely related to some toxic factor than to hypertension. In fact hypertension only arises in the majority of cases when destruction of the kidneys by vascular changes has resulted in renal insufficiency. The inflammatory lesions in the vessels predominate and endarteritis, as Volhard points out, is irregular in distribution. Hypertension of the malignant type occurs in relatively few instances and in these some additional factor appears to be present; thus antecedent hypertension was present in 2 cases in the literature and in 2 others a history of lead poisoning was obtained.

We are led to the conclusion that the etiological agent (allergic or otherwise) which produces the inflammatory lesions in the arteries in periarteritis nodosa is not *per se* responsible for the malignant character of the hypertension but only when acting on arteries which show an abnormal reactivity. We were previously led to postulate a difference in reactivity of the arteries to explain the different character of malignant hypertension in young and old subjects; and we suggested above that the blood vessels might be "sensitized" by an attack of acute diffuse glomerulonephritis. The whole evidence leads us to the conclusion that two factors are necessary for the development of malignant nephrosclerosis — a preëxisting hyperactivity or "sensitization" of the arteries on which is superimposed some precipitating factor, allergic or otherwise.

#### SUMMARY AND CONCLUSIONS

(A) Benign hypertension and benign nephrosclerosis may show a parallel development but in the early stages are not causally related. In the later stages, however, there may be a reciprocal relationship, *i.e.*:

(1) Hypertension acts as an accelerating factor on the development of arteriosclerosis.

(2) Arterial and arteriolar sclerosis of the kidney, when severe enough to produce impairment of renal function, may give rise to

"renal" fixation of the hypertension. Such cases are termed "de-compensated benign nephrosclerosis" since clinical and histological evidence shows that the impairment of function is of true renal origin.

(B) Malignant hypertension and malignant nephrosclerosis, on the other hand, show a definite correlation.

(1) On clinical and histological grounds malignant hypertension is to be regarded as a primary generalized vascular disease of which malignant nephrosclerosis represents the "renal end-stage." Cases are described in which death occurs from malignant hypertension before the renal end-stage is reached.

(2) When malignant hypertension progresses to the stage of malignant nephrosclerosis, the condition is clinically and histologically characteristic, as described by Volhard and Fahr. The main objection to their classification is the existence of so-called "borderline" cases, which are neither clinically nor histologically characteristic. Of these cases, in our interpretation, one group consists of cases of malignant hypertension in which death occurs before the renal phase develops, the other group comprises older subjects in whom the malignant hypertension is less fulminant and may be superimposed on benign nephrosclerosis.

(3) Endarteritis in its diffuse form is regarded as the most characteristic histological sign of malignant hypertension. Arteriolitis (arteriolar necrosis) is more closely related to the terminal renal failure than to the hypertension itself.

(4) Various hypertensive states may act as precursors of malignant hypertension. Evidence is presented that diffuse glomerulonephritis may similarly be associated with or followed by malignant hypertension, thereby explaining the occurrence of the "specific" vascular lesions in the kidney in diffuse glomerulonephritis.

(5) A study of the relation of periarteritis nodosa to malignant nephrosclerosis provides suggestive evidence that two factors are necessary for the development of malignant hypertension, namely, a preëxisting hyperactivity or "sensitivity" of the arteries, on which is superimposed a precipitating factor, allergic or otherwise.

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## DESCRIPTION OF PLATES

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### PLATE 7

- FIG. 1. Fibrinoid degeneration in the small vessel staining dark purplish blue with eosin-methylene blue. Wall of large vessel shows hyalinization only. Stains red.
- FIG. 2. Similar fibrinoid degeneration showing irregular, flake-like appearance of fibrinoid material in hyaline mass. Eosin-methylene blue.
- FIG. 3. Early alternative glomerulitis showing necrosis of swollen epithelial cells with adhesion to Bowman's capsule at this point. Eosin-methylene blue.



I



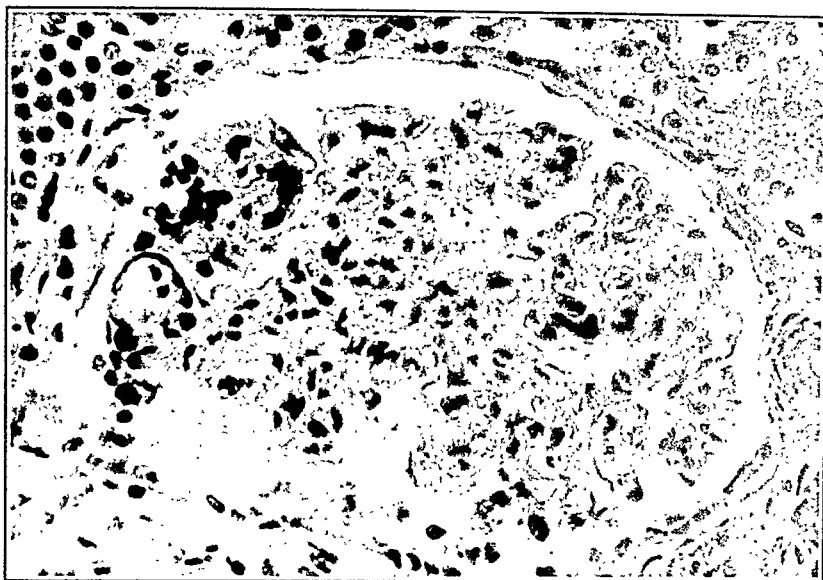
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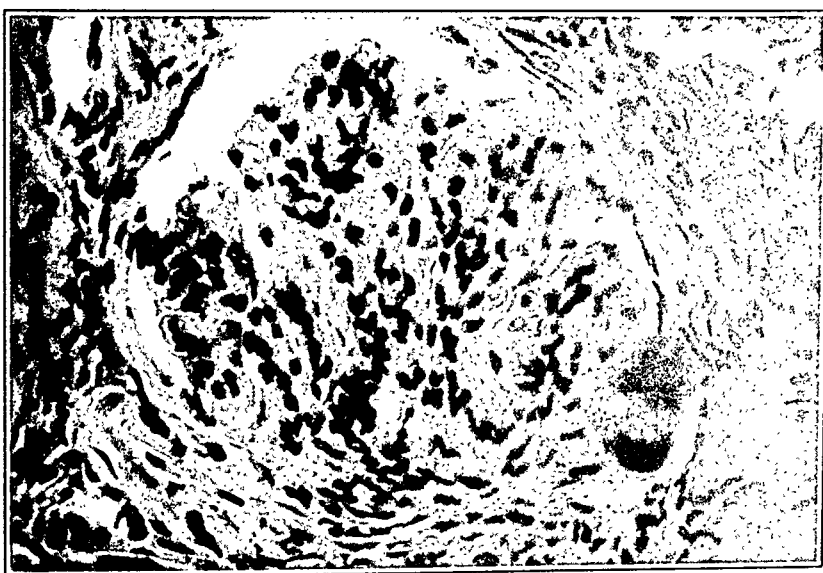
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PLATE 8

- FIG. 4. Early alterative glomerulitis; several loops show necrotizing processes. There seems to be a slight increase of nuclei in these areas. Eosin-methylene blue.
- FIG. 5. Alterative glomerulitis showing acute necrosis at one point (dark area which stains purplish). Several old adhesions. Eosin-methylene blue.
- FIG. 6. Extensive necrotizing glomerulitis; several loops involved simultaneously. Eosin-methylene blue.



4



5



6



# INTERCAPILLARY LESIONS IN THE GLOMERULI OF THE KIDNEY \*

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## INTRODUCTION

Since the existence of an intercapillary connective tissue, not only confined to the hilum but also extending to the periphery, has been definitely recognized in the normal glomerulus,<sup>1, 2, 3</sup> more attention has been paid to the pathological changes in this connective tissue framework. MacCallum, in particular,<sup>4</sup> has recently analyzed the changes in the intercapillary connective tissue which are associated with various pathological conditions in the kidney. He describes edema, amyloid degeneration, hyalinization and growth of the connective tissue. Of special interest in relation to the material we present is his statement that intracapillary glomerulonephritis consists in an increase of intercapillary connective tissue. For this reason he suggests the term "intercapillary" in place of "intracapillary" glomerulonephritis. From his description it is not quite clear whether he restricts the term "intracapillary glomerulonephritis" to the cases so-called by Fahr<sup>5</sup> or includes also the common "extracapillary glomerulonephritis."

One of us<sup>6</sup> has recently described the frequently observed broadening of the connective tissue of the glomerulus as an aging process which apparently develops independently of hypertension and arteriosclerosis. Further studies show that in a certain group of cases this change may so dominate the histological picture as to give a characteristic appearance. Since the clinical findings may be equally characteristic, it seems justifiable to describe these cases as a special group. In attempting to do so, however, considerable difficulties are encountered, especially in advanced cases, in differentiating them from so-called intracapillary glomerulonephritis (Fahr). We have, therefore, contrasted the features of this special group with those of

\* Received for publication August 10, 1935.

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true intracapillary and extracapillary glomerulonephritis. A detailed study of the basement membrane enables us to make a definite distinction in the majority of cases.

### STAINING METHOD

1. Lithium carmine  $1\frac{1}{2}$  hours at  $55^{\circ}$  C.  
(Stain contains  $2\frac{1}{2}$  gm. carmine in 100 cc. saturated lithium carbonate.)
2. Without contact with water transfer to 1 per cent hydrochloric acid in 70 per cent alcohol for 3 minutes. Change once.
3. Wash with distilled water.
4. 1 per cent phosphomolybdic acid 30 seconds.
5. Wash with distilled water once.
6. Aniline blue-orange G stain  $\frac{3}{4}$  hour at  $55^{\circ}$  C.  
(0.5 gm. aniline blue with 2 gm. orange G in 100 cc. 1 per cent phosphomolybdic acid. Stir well and allow to stand a few hours. Filter.)
7. Wash with distilled water several times.
8. 1 per cent phosphomolybdic acid 45 seconds.
9. Wash with distilled water once.
10. Differentiate in 95 per cent alcohol to which is added 1 cc. 15 per cent sodium hydroxide per 100 cc. until first traces of reddish stain appear.
11. Wash quickly in excess of 95 per cent alcohol. The stain washed out turns blue. Continue until no further blue appears.
12. Absolute alcohol, xylol, balsam.

Fresh Zenker-fixed material must be used. Paraffin embedding and thin sections are necessary. If the section is not differentiated enough it may be brought back to alkaline alcohol for a very short time.

*Results:* Connective tissue and basement membranes deep blue, nuclei red with clear structure. Cytoplasm grayish pink, hyaline droplets blue. Red blood cells golden yellow or occasionally greenish. The colors are more delicate than in the Heidenhain stain and details of the cytoplasm and basement membrane are clearly brought out.

## A. INTERCAPILLARY GLOMERULOSCLEROSIS

The term intercapillary glomerulosclerosis has been applied to the group of cases under discussion, of which we present 8 examples showing different stages of the glomerular lesion.

**CASE 1.** B. P., female, aged 62 years, on admission complained of shortness of breath and drowsiness. There was a 3 years history of diabetes with 2 months known hypertension.

*Physical Examination:* The patient was semicomatose with signs of cardiac failure, and generalized edema including arms, face and neck was present. Blood pressure 180/100 mm. Hg.

*Urine:* Specific gravity 1004-1010; large trace of albumin; many leukocytes in sediment; sugar negative.

*Blood:* Non-protein nitrogen 58 mg. per 100 cc.

*Autopsy Findings*

*Anatomical Diagnosis:* Myocardial failure.

*Gross Appearance of Kidneys:* Combined weight 300 gm. Average in size. The capsule strips with difficulty from a granular reddish brown surface with occasional stellate scars. On section the cortex averages 5 mm. in thickness, is reddish brown with normal striations and clearly demarcated from the medulla.

**CASE 2.** H. G., male, aged 60 years, was admitted in extremis complaining of pain in the chest and cough. No previous history was obtainable.

*Physical Examination:* Generalized edema including the hands and face was present. Blood pressure 160/70 mm. Hg. Signs of bronchopneumonia and cardiac failure were present and the patient died 3 hours after admission.

*Autopsy Findings*

*Anatomical Diagnoses:* Chronic nephritis, bronchopneumonia.

*Gross Appearance of Kidneys:* Combined weight 380 gm. The capsule strips with ease, leaving a finely granular surface. On section the cortex is pale grayish purple, 5 mm. in thickness, with grayish white medulla.

**CASE 3.** A. T., female, aged 63 years, was admitted with a complaint of gradually increasing edema for 10 months with shortness of breath on exertion. There was a previous history of diabetes.

*Physical Examination:* Edema of legs, thighs, abdomen, arms and face was present. Blood pressure 120/80 mm. Hg.

*Urine:* Specific gravity 1017-1024; large trace of albumin; sugar ++.

*Autopsy Findings*

*Anatomical Diagnoses:* Myocardial failure, arteriosclerosis and diabetic nephrosis.

*Gross Appearance of Kidneys:* Combined weight 240 gm. The capsule strips with difficulty from a grayish brown, very finely nodular surface containing several smooth depressed scars. Cut surface reveals a cortex 3-5 mm. broad, grayish brown in color, and clearly demarcated from darker red medulla.



CASE 4. M. H., female, aged 51 years, was admitted in a semicomatose condition with twitching of muscles, vomiting and shortness of breath. There was a 10 years history of diabetes.

*Physical Examination:* Edema of ankles, bilateral cataract and severe arteriosclerosis were present. Blood pressure 190/80 mm. Hg.

*Urine:* Specific gravity 1012; large trace of albumin; many leukocytes and occasional red cells in sediment.

*Blood:* Non-protein nitrogen 170 mg. per 100 cc.

#### *Autopsy Findings*

*Anatomical Diagnoses:* Generalized arteriosclerosis, bronchopneumonia.

*Gross Appearance of Kidneys:* Combined weight 230 gm. The capsule strips easily leaving a yellowish gray, coarsely granular surface. On section a very pale grayish yellow, finely granular cortex 5 mm. thick, with pale grayish red medulla is seen. Marked atheroma and calcification of renal vessels is present.

CASE 5. L. S., female, aged 48 years, was admitted with a complaint of swelling of legs and blurring of vision. There was a 3 years history of diabetes.

*Physical Examination:* The patient was stuporous with Cheyne-Stokes respiration and generalized edema was present. There were retinal exudates and hemorrhages. Blood pressure 230/110 mm. Hg.

*Urine:* Large trace of albumin; sugar +.

*Blood:* Non-protein nitrogen 133 mg. per 100 cc.; blood sugar 275 mg. per cent.

#### *Autopsy Findings*

*Anatomical Diagnoses:* Generalized anasarca, myocardial failure.

*Gross Appearance of Kidneys:* Combined weight 370 gm. The surface is granular. On section the cortex is 6 mm. thick, and yellowish gray-white in color.

CASE 6. A. H., female, aged 49 years, was admitted with a 2 weeks history of vomiting and oliguria. There was a 15 years history of diabetes. Right nephrectomy for renal calculi was performed 18 years before.

*Physical Examination:* The patient was comatose with Cheyne-Stokes respiration.

*Urine:* Albumin present; many leukocytes in sediment; sugar ++.

*Blood:* Non-protein nitrogen 150 mg. per 100 cc.

#### *Autopsy Findings*

*Anatomical Diagnoses:* Generalized arteriosclerosis, chronic nephritis.

*Gross Appearance of Kidney:* Weight 280 gm. (left). The capsule strips with difficulty leaving a coarsely granular surface. On section the cortex is grayish red with multiple irregular scars scattered throughout and poorly demarcated from the medulla.

CASE 7. C. H., male, aged 68 years, was admitted complaining of dyspnea on exertion and edema of feet. There was a 6 years history of diabetes.

*Physical Examination:* Edema of ankles was present. Blood pressure 230/110 mm. Hg.

*Urine:* Specific gravity 1010-1022; large trace of albumin; sugar +.

*Blood:* Non-protein nitrogen 25 mg. per 100 cc.

#### *Autopsy Findings*

*Anatomical Diagnoses:* Hypernephroma, pulmonary infarction.

*Gross Appearance of Kidneys:* The right kidney weighs 375 gm. The capsule strips easily leaving a smooth, pale, swollen surface. On section the cortex is 7 mm. broad and appears pale and swollen. The left kidney contains a hypernephroma.

CASE 8. A. F., male, aged 58 years, was admitted complaining of increasing swelling of the feet, legs and abdomen. There was a 5 years history of diabetes.

*Physical Examination:* Bilateral cataract present. Pitting edema of legs and abdomen and marked ascites. Blood pressure 190/100 mm. Hg.

*Urine:* Specific gravity 1007-1015; large trace of albumin; many leukocytes and red cells in sediment.

*Blood:* Non-protein nitrogen 87 mg. per 100 cc.

#### *Autopsy Findings*

*Anatomical Diagnoses:* Generalized atheroma, myocardial failure.

*Gross Appearance of Kidneys:* Combined weight 400 gm. The capsule strips with difficulty from a finely granular surface. The cut surface is mottled gray and yellow. The cortex is 6 mm. broad, well demarcated from the medulla.

#### *Microscopic Appearances in the Kidney*

(1) *The Glomeruli:* The most striking feature is the great regularity with which the hyalinization of the glomerulus is confined to its center, or even to the center of one lobule (Fig. 1). With the eosin-methylene blue stain an entirely homogeneous mass is seen, suggestive of amyloid, but negative reactions are obtained with all the amyloid stains. Fat stains (Sudan III) usually give a homogeneous pinkish color, while double refraction is only exceptionally present in smaller circumscribed areas. Toward the periphery of the glomerulus the basement membrane of the capillaries seems to emerge from the hyaline mass but its outline is sharply defined and usually surrounds a widely patent lumen (Fig. 2). The basement membrane may be delicate like the normal one, or somewhat thickened, but is never wrinkled or split (Fig. 3). The number of capillaries is apparently reduced — in many cases there remains only a ring of open capillaries surrounding the central hyaline mass (Fig. 1). The remainder appear to have been buried in it and to have disappeared.

The hyaline mass itself obviously represents a broadening of the intercapillary connective tissue. This can be observed particularly

well at the hilum. A very high degree of arteriosclerosis with fatty degeneration of the arterioles is present in most of the cases and the hyaline material is seen to be continuous from the vasa afferentia into the intraglomerular mass as it extends from the center of the lobules to the periphery (Fig. 4). The intercapillary hyalinization is not, however, to be regarded merely as an extension of the degenerative process from the vas afferens since it is found in glomeruli where the vas afferens is normal. Not infrequently pictures are encountered similar to those recently described by one of us as an "aging process of the glomerulus," but the axial thickening is much more massive and striking. It must be emphasized, however, that there is only a difference in degree between the less marked changes frequently observed in senile kidneys and the lesion here described. Although the basement membrane of the capillaries seems to be well preserved for a long time, the change gradually extends to the periphery. This mode of extension is most characteristic. Eventually the capillary walls thicken homogeneously and near the central hyaline mass they collapse and finally merge with the central hyalin (Fig. 5). It is also possible, as MacCallum points out, that the capillaries are partly pushed toward the periphery and preserved there for a long time.

While the capillaries are fusing with the central hyalin, the nuclei, especially those of the endothelial cells, appear well preserved and are crowded together (Fig. 6). This process may be observed step by step: the lumen of the capillaries becomes a narrow slit, the endothelial nuclei increase in density but retain their elongated form and are finally entirely embedded in a homogeneous hyaline mass (Fig. 7). This may give rise to an apparent increase in nuclei which simulates an insidious proliferative process, thereby leading to misinterpretations, as we shall see later. The nuclei are often arranged in "onion layers" at the periphery of the hyalin (Fig. 6), presumably originating from the endothelial cells of the collapsed capillary loops. Two reasons prevent one from attributing this appearance to true proliferation: (1) Immature nuclei and mitoses are never seen — on the contrary the nuclei are almost invariably pyknotic and in a state of necrobiosis; (2) The mode of development of the apparent increase in nuclei can be seen to be the result of a regressive process. There is, furthermore, no definite proof of an inflammatory process. Regarding the origin of the nuclei many observations have led to the con-

viction that sometimes at least they originate from the endothelial cells of collapsed capillaries. Whether in addition there is a true "growth" of interstitial tissue as MacCallum assumes, it is difficult to decide. The more centrally situated nuclei lie in a dense homogeneous mass which certainly originates from the intercapillary connective tissue, but in later stages the capillaries with their endothelial and epithelial cells become embedded, and the origin of the nuclei cannot be recognized.

(2) *Capsular Changes*: Severe changes also occur in the glomerular capsule. A substance is deposited which at first appears translucent with a slightly pinkish stain, and later becomes more homogeneous, hyalin-like, and often contains abundant lipoid. The mass lies between the basement membrane and the epithelial layer of Bowman's capsule lifting the epithelial cells (Fig. 8). It may be deposited in such quantity that the capsular space is greatly narrowed (Fig. 9). A connective tissue reaction appears in the outer layers in later stages only — apparently a resorptive or organizing process. Broadening of the connective tissue occurs by the formation of concentric layers of fibrils and nuclei. The mode of development of this capsular change, which frequently accompanies the glomerular lesion, definitely indicates the primary degenerative character of the whole process.

The intercapillary process described above is practically diffuse, in the sense that almost all the glomeruli are affected. Although the severity of the lesion may vary, in a few cases it is the only lesion which can be found. In others, however, a scanty focal glomerulitis is present of the type we have described in decompensated benign nephrosclerosis and malignant nephrosclerosis. There are, in these instances, as in decompensated benign nephrosclerosis, definite signs histologically and clinically of renal insufficiency and we interpret these cases as decompensated benign or malignant nephrosclerosis complicated by intercapillary glomerulosclerosis.

The tubular changes have no special significance. The degenerative processes common to all arteriosclerotic processes are found. There is, however, in most cases a striking deposition of fat and doubly refracting lipoid in the tubules and in the interstitial tissue. No diagnostic significance, however, is attached to lipoid deposition in our cases since the amount was never sufficient to give the gross appearance of a lipoid nephrosis. Moreover, many cases of severe

uncomplicated arteriosclerosis of the kidneys show a fairly high degree of lipoid infiltration of the tubules and interstitial tissue. One case grossly appeared to resemble a nephrosis but lipoid deposition was not extraordinary, the tubules showing chiefly albuminuric and hyaline droplet degeneration.

### *Comment*

*The Clinical Picture:* The separation of the above group of cases, especially from intracapillary glomerulonephritis, has been made on histological grounds which are discussed later. On reviewing the clinical data it was surprising to find a previous history of diabetes in all these cases, with the exception of one (Case 2) in which death occurred 3 hours after admission and no history was obtainable.\* It must be emphasized that only a small proportion of cases of diabetes appears to show this lesion at autopsy. A second striking feature was severe and widespread edema. The clinical picture appears in fact to be almost as characteristic as the histological one: the patients are relatively old; hypertension is present, usually of the benign type, and the kidneys frequently show signs of decompensation; there is a history of diabetes usually of long standing; the presenting symptoms may be those of edema of the nephrotic type, renal decompensation or heart failure; the urine contains large amounts of albumin and there is usually impairment of concentrating power with or without nitrogen retention.

Although some degree of cardiac failure is frequently present, the edema is out of all proportion to this and may be extreme when no signs of heart failure can be demonstrated. Furthermore, its generalized distribution, especially its extension to the arms and face, leads us to regard it as nephrotic rather than cardiac in type, and this conclusion is supported by the constant finding of severe albuminuria.

*The Pathological Picture:* The gross appearance of the kidneys is not characteristic. They present the picture of arteriosclerotic contraction which may be in part or completely obscured by the signs of

\* Since the investigation was completed several other instances of intercapillary glomerulosclerosis have been encountered. All were cases of diabetes except one in which a questionable reducing reaction was present in the urine, but no information was available in regard to the previous history.

nephrosis, *i.e.* they may be enlarged and swollen with grayish or yellowish external and cut surfaces.

The histological picture is, however, very characteristic. Arteriosclerosis is present, usually of very high degree, fatty degeneration of the arterioles being unusually conspicuous. Intercapillary hyaline change is discernible in most of the glomeruli even under low power. The special stain is necessary to demonstrate the earlier stages of the process but when this is used the lesion cannot be overlooked. All degrees of this hyaline change can be observed down to those produced simply by the aging process, and the cases described above represent an extremely severe type.

The tubules very often show fatty degeneration, and lipoid is frequently found in the interstitial tissue.

In addition to these characteristic features the signs of true renal decompensation we have previously described are commonly found, and when this is the case the capsular adhesions though focal in distribution may be surprisingly frequent.

### B. INTRACAPILLARY GLOMERULONEPHRITIS (FAHR)

We have referred to the great difficulties which may arise in differentiating late stages of intercapillary glomerulosclerosis and so-called intracapillary glomerulonephritis. The latter differs from the common extracapillary glomerulonephritis in the absence or very scanty occurrence of capsular proliferation. In the subchronic stage, intracapillary glomerulonephritis is frequently complicated by the "nephrotische Einschlag." Histologically a peculiar hyalinization of the glomerulus occurs which accentuates its lobulation in the same way as in intercapillary glomerulosclerosis (Fig. 10). In the early stages abundant leukocytes appear in the capillaries and emigrate into the capsular space and convoluted tubules. In the later stages the only finding may be an increase in nuclei which Fahr interprets as an insidious endothelial proliferation. He believes that hyalinization is due to fusion of the thickened capillary walls. Adhesions are frequently found and thickening of the connective tissue of Bowman's capsule is often present. During the earlier stages there is little difficulty in diagnosing the inflammatory nature of the disease on account of the presence of leukocytic infiltration. In the chronic stages, however, where leukocytic infiltration is almost absent, capsular ad-

hesions may occur only focally and a definite decision may be very difficult. True endothelial proliferation — or at least increase in endothelial nuclei — can sometimes be recognized, but such a finding is inconstant; in general the apparent increase in nuclei cannot be regarded as proof of an inflammatory process. Pyknotic nuclei are embedded in a hyaline mass and it may be impossible to say whether they originate from endothelial or connective tissue cells and whether there is an actual increase in nuclei or merely a crowding effect. The clinical evidence is frequently negative since the acute stage of the glomerulonephritis may pass unnoticed. Intercapillary glomerulosclerosis may, in fact, so resemble intracapillary glomerulonephritis in the late stages that a distinction is impossible. We encountered one such case.

Detailed examination of cases of intracapillary glomerulonephritis shows that in most glomeruli the main mass of hyalin is localized at the center of the lobules; and as in intercapillary glomerulosclerosis a ring of open capillaries is left at the periphery. Although red blood cells are frequently seen in these peripheral capillaries, the lumen is rather narrow and never distended. The hyalinization is easily distinguished from that which occurs in the more common extracapillary glomerulonephritis. In this condition there is irregular thickening of the capillary loops and the lumen is narrowed by the broadening and splitting of the basement membrane. The process often affects the glomerulus focally leaving the unaffected parts free. Moreover, the hyalinization tends to start in the periphery, whereas in intracapillary glomerulonephritis it begins at the center of the lobule.

The central origin of hyalinization in intracapillary glomerulonephritis with preservation of the peripheral capillaries indicates that the degeneration of the connective tissue framework occurs independently of and is superimposed on hyalinization of the capillary wall. If the latter alone were present we should expect the hyalinization of the glomerulus to be diffusely distributed, since in the earliest stages of the disease all the capillaries are involved. The following observation gives more definite proof that hyalinization of the intercapillary tissue occurs as an independent process. In some cases one encounters occasional glomeruli which are not involved in the otherwise diffuse inflammatory lesion and which show hyalinization of the intercapillary connective tissue framework without any change in the peripheral zone. In some of these instances a marked swelling

and hyaline droplet degeneration of epithelial cells is present but in others the glomerulus is normal except for the change in the intercapillary tissue (Fig. 11).

It has already been emphasized that the apparent increase in nuclei in the hyaline mass in intracapillary glomerulonephritis may be attributed to crowding of the endothelial nuclei of collapsed capillaries and does not furnish proof of an inflammatory process. Changes occur, however, in the basement membrane of the preserved peripheral capillaries which in our experience are characteristic of such a process. These are blurring of outline and splitting of the basement membrane, which are also commonly found in extracapillary glomerulonephritis (Fig. 12). The development of intracapillary hyaline fibers, described by McGregor, is rare in the intracapillary form.

Diffuse involvement of the capillaries by these changes definitely indicates a primary diffuse lesion of the glomerular capillaries in contradistinction to intercapillary glomerulosclerosis, even though no actual inflammatory infiltration may be demonstrated. A marked swelling of epithelial cells, which is found even in the later stages, further indicates the inflammatory nature of the process and when diffusely distributed may be regarded as a diagnostic sign. The same change is found in intercapillary glomerulosclerosis but is focal in distribution.

The capsular changes closely resemble those of intercapillary glomerulosclerosis. The same formation of connective tissue layers is present without, however, any epithelial proliferation (crescent formation). Volhard<sup>7</sup> considers this as a reaction to waste products which diffuse outwards from the capsular space. Fahr believes the process to be inflammatory, in keeping with the "insidious endothelial proliferation" in the glomerulus. One can, however, only state that the same capsular change occurs in association with a purely degenerative process in intercapillary glomerulosclerosis, and hence does not necessarily indicate an insidious inflammatory reaction.

### *Comment*

In intracapillary glomerulonephritis capsular proliferation (crescent formation) is inconspicuous, but a most characteristic feature is the massive degeneration of the connective tissue framework of the glomerulus which complicates the capillary lesion. Blurring and



splitting of the basement membrane are regarded as indicative of the inflammatory nature of the process even in the late stages when cellular infiltration and proliferation are absent, and this capillary lesion differentiates the condition from intercapillary glomerulosclerosis. It is noteworthy that in cases of both arteriosclerosis and intracapillary glomerulonephritis the intercapillary degeneration tends to be accompanied by fatty tubular nephrosis and interstitial deposition of doubly refracting lipid.

### C. EXTRACAPILLARY GLOMERULONEPHRITIS

The changes in the basement membrane of the peripheral capillaries in intracapillary glomerulonephritis are also found in the extracapillary form. Our observations are based on McGregor's<sup>8</sup> detailed description of structural changes in the glomerulus in glomerulonephritis which are brought out by combined nuclear and basement membrane stains. She claims that a most characteristic sign is the development of intracapillary hyaline fibers, either from fibrin threads or from the basement membrane itself. Hyalinization of the glomerulus is assumed to result from thickening and fusion of these fibers. Our observations essentially confirm these findings with the following additions:

(1) Certain changes in the basement membrane are found in inflammatory lesions of the glomeruli and are no less characteristic than intracapillary hyaline fibers. They may in fact be present in the absence of the latter. Such changes include splitting of the basement membrane which appears blurred and definitely thickened, though not wrinkled. The membrane appears as if teased out into a meshwork of delicate fibers which narrow the lumen. Where the fibers are thick and the section cuts a loop tangentially, they give the impression of being intracapillary. It cannot be stated whether or not all the intracapillary fibers are to be explained in this way; some may well be actually split off the basement membrane. In any case the distribution of both intracapillary fibers and splitting of the basement membrane is irregular throughout the glomerulus. It may be diffuse or confined to small areas, but the peripheral loops are as severely involved as the more central ones. MacCallum states that he has not observed intracapillary occlusions in "intracapillary glomerulonephritis." This holds true for intracapillary glomerulone-

phritis in Fahr's sense, but in the common (extracapillary) form our observations support McGregor's contention that such occlusions are of frequent occurrence.

(2) In extracapillary glomerulonephritis there is frequently, in addition to the above changes, thickening of the intercapillary connective tissue. The points of difference from the similar lesion in intracapillary glomerulonephritis have been outlined above. In particular, the thickening is irregular and focal in its distribution throughout the glomerulus, and does not appear to have any characteristic significance.

### *Comment*

In the common or extracapillary glomerulonephritis thickening of the intercapillary connective tissue is irregular or focal in distribution and is relatively insignificant. Intracapillary fibers, which are found in all inflammatory lesions of the glomerulus, are associated with an equally characteristic change, namely, broadening and splitting of the capillary basement membrane.

### DISCUSSION

The glomerular changes in glomerulonephritis include two characteristic elements: (1) alteration of the basement membrane leading to "intracapillary" fibrillation; and (2) a purely degenerative process, the deposition of intercapillary hyaline material. This intercapillary glomerulosclerosis is apparently an independent lesion since it may be superimposed on pure arteriosclerosis as well as on inflammatory changes in the glomeruli. It may therefore be considered as a "complication" of glomerulonephritis.

It has been shown that intercapillary glomerulosclerosis and intracapillary glomerulonephritis have in common a degenerative process in the intercapillary connective tissue. They present also a striking clinical similarity in their frequent association with the nephrotic type of edema — the "nephrotische Einschlag," which has been attributed to a general metabolic disorder. The invariable finding of diabetes in cases of pure intercapillary glomerulosclerosis lends support to such a theory.

## SUMMARY AND CONCLUSIONS

Cases are described which show a striking hyaline thickening of the intercapillary connective tissue of the glomerulus. Evidence is presented which indicates that the change is degenerative in nature and suggests that arteriosclerosis and diabetes may play a part in its causation. The lesion is therefore termed intercapillary glomerulosclerosis. The characteristic clinical features are a previous history of diabetes, severe and widespread edema of the nephrotic type and gross albuminuria. Hypertension is frequently present, in many cases associated with renal decompensation.

The same histological picture frequently complicates intracapillary glomerulonephritis but in the later stages this condition is differentiated histologically by blurring and splitting of the capillary basement membrane.

In extracapillary glomerulonephritis thickening of the intercapillary connective tissue is relatively insignificant and the basement membrane changes are more pronounced.

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8. McGregor, L. The cytological changes occurring in the glomerulus of clinical glomerulonephritis. *Am. J. Path.*, 1929, 6, 559-585.

## DESCRIPTION OF PLATES

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### PLATE 9

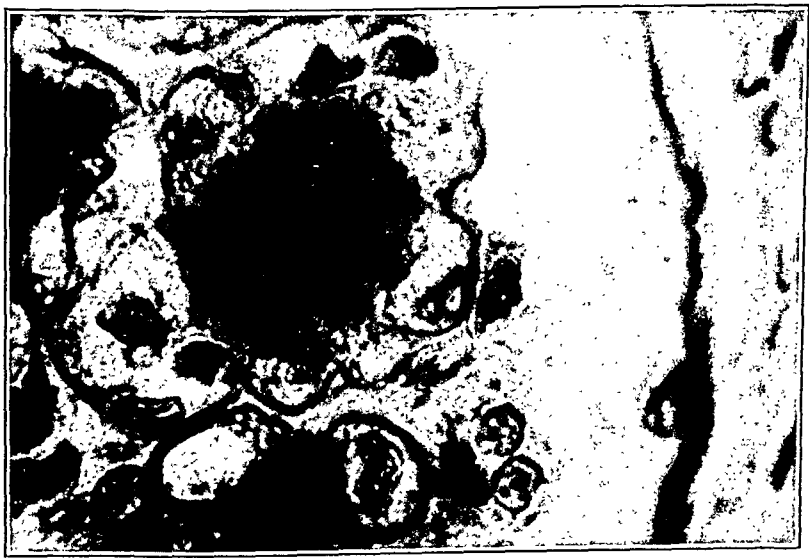
- FIG. 1. Inter-capillary glomerulosclerosis. Central hyalinization of all glomerular loops. Peripheral capillaries patent. Special basement membrane stain.
- FIG. 2. Central hyalinization of peripheral loop. Capillaries wide open and contain red blood cells. Basement membrane clearly delineated and delicate. Special basement membrane stain. High power.
- FIG. 3. Inter-capillary hyalinization. Peripheral capillaries patent, nuclei of endothelial and epithelial cells clearly recognizable. Capillary basement membrane somewhat thickened. Special basement membrane stain. High power.



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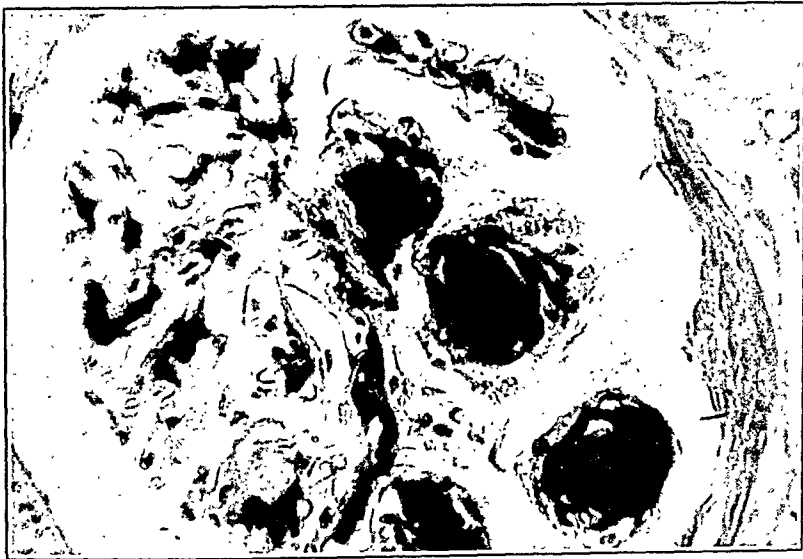
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PLATE 10

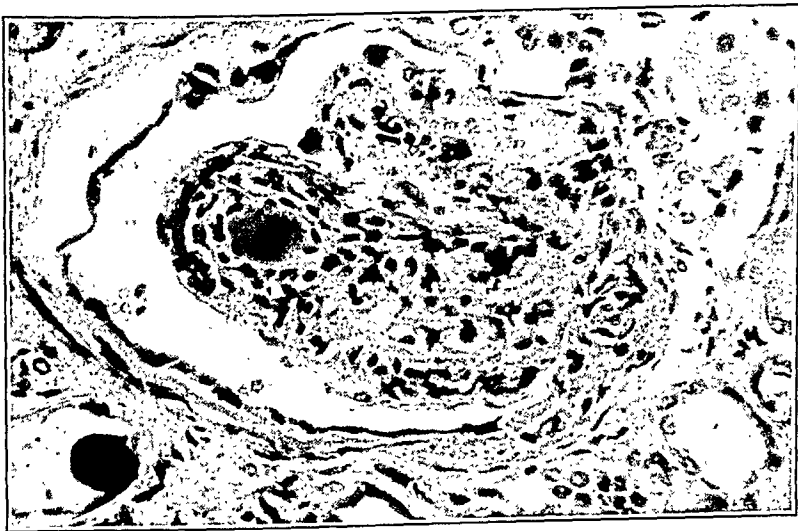
- FIG. 4. Hyalinization of intercapillary connective tissue extending into most of the loops, in direct continuity with the hyaline material of the vas afferens. Special basement membrane stain. Medium power.
- FIG. 5. Intercapillary hyalinization in several loops; the hyaline material encroaches upon the capillary wall which is homogeneously thickened. The capillaries are collapsed and their lumen reduced to a narrow slit. Special basement membrane stain. Medium power.
- FIG. 6. Central hyalinization clearly seen even with eosin-methylene blue stain. Crowding of endothelial nuclei around collapsed capillaries gives appearance of "onion layers." Eosin-methylene blue stain. High power.



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## PLATE II

FIG. 7. Well preserved endothelial nuclei are seen embedded in central hyaline mass. Special basement membrane stain.

FIG. 8. Hyaline fatty mass seen between basement membrane and epithelial cells of Bowman's capsule. Special basement membrane stain. High power.

FIG. 9. Sudan III fat stain shows large fatty mass between epithelial cells and basement membrane of Bowman's capsule. The picture also shows fat in the vas afferens, some fatty degeneration of capillary loops and fat in the tubular epithelial cells. Sudan III stain. High power.



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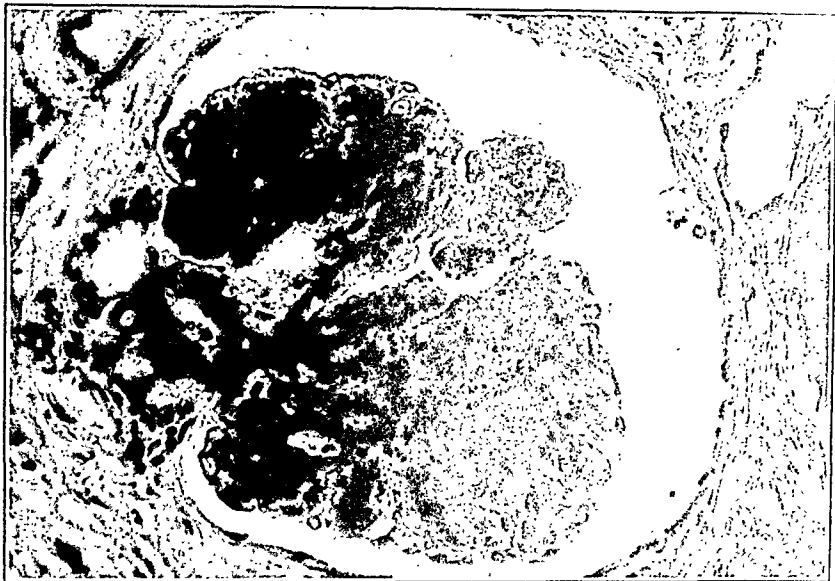
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PLATE 12

FIG. 10. Intracapillary glomerulonephritis. Central hyalinization identical in situation with that of intercapillary glomerulosclerosis (see Fig. 1). Notice open capillaries in the periphery. Special basement membrane stain. High power.

FIG. 11. Single glomerulus in an otherwise diffuse intracapillary glomerulonephritis. There is no other change but a severe central intercapillary hyalinization. Special basement membrane stain. High power.

FIG. 12. Intracapillary glomerulonephritis showing blurred outline of peripheral capillaries. Special basement membrane stain. High power.



10



11



12



# INFLAMMATORY LESIONS IN THE GLOMERULI IN PYELONEPHRITIS IN RELATION TO HYPERTENSION AND RENAL INSUFFICIENCY \*

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Inflammatory lesions in the glomeruli in cases of pyelonephritis are relatively uncommon even when contraction of the kidney is extreme. When they occur, moreover, they are focal in distribution. In a previous communication <sup>1</sup> we have described in detail inflammatory changes in the glomeruli in "essential" hypertension. Such glomerulitis is also focal in distribution and is encountered in cases which have progressed to the stage of renal insufficiency, *i.e.* in malignant nephrosclerosis and in benign nephrosclerosis with "renal decompensation." We came to the conclusion that glomerulitis in these conditions is the result of combined toxic and ischemic factors. It therefore seemed of importance to study the relation of focal glomerulitis to hypertension and renal insufficiency in a disease where the renal damage results from changes in the tubules and interstitial tissue independent of the vascular elements of the kidney.

## LITERATURE

Reports in the literature on the glomerular lesions in pyelonephritis are scanty. Putschar in Henke and Lubarsch's Handbook (p. 430) says "the glomeruli appear in the acute stages (of pyelonephritis) to be practically uninvolved." Russell,<sup>2</sup> however, describes in some detail glomerular changes in "ascending" and "interstitial" nephritis. In the former invasion of the capsular space and of the glomerular tuft by neutrophil leukocytes and desquamation of the capsular epithelium were observed in the early stages. In later stages adhesive glomerulitis and slight proliferative glomerulitis were found, accompanied in still more chronic cases by focal necroses in the tufts. In "interstitial" nephritis two main types of glomerular change were observed in the chronic stages; first, focal necroses and adhesions resembling those seen in "Bright's disease"; and second,

\* Received for publication August 14, 1935.

† Rockefeller Traveling Fellow.

concentric proliferation of fibroblasts in the periglomerular tissues, occasionally associated with proliferative capsulitis or more rarely with slight proliferative glomerulitis. Subsequently hyalinization of the capsule occurs with adhesions to the glomerular tuft. Staemmler and Dopheide<sup>3</sup> and later Pfeiffer<sup>4</sup> describe two types of glomerular lesions in ascending contraction of the kidney; first, a primary thickening of the capsule leading to hyalinization of the glomerulus; and second, focal glomerular adhesions. These are alleged to follow focal desquamation of epithelial cells. Hyalinization of the glomerular loop and finally of the whole glomerulus results and granulation tissue invades the hyaline mass from without. Eventually the affected glomeruli disappear entirely. The authors regard the process as due to a toxin diffusing through the glomerular capsule from without.

### SELECTION OF CASES

Fifty-six cases of pyelonephritis were studied from consecutive autopsies performed during 1932, 1933 and 1934 (Tables I and II).<sup>\*</sup> Twenty-nine of these were associated with primary lesions in the genito-urinary tract (prostatic enlargement with urinary retention 11, calculous pyelonephritis 5, prostatic abscess 4, urethral stricture 3, retention of neurogenic origin 3, carcinoma of bladder 1, vesico-vaginal fistula 1, and bilateral hydronephrosis of unknown origin 1). In 26 of these cases bilateral pyelonephritis was present, in 2 the lesion was unilateral.

Of the remaining 27 cases 10 showed gross changes in the ureters, pelves and calyces, *e.g.* dilatation of the pelvis with roughening, thickening and injection of the mucosa. Such signs were absent in 17 cases. These showed evidence, however, of active inflammation and the chronicity of the process was judged by the degree of renal contraction. In the chronic cases of this group a diagnosis of ascending infection could not be made definitely but the diagnosis of pyelonephritis was made on the basis of clinical, macroscopic and microscopic evidence discussed elsewhere.<sup>1</sup> Macroscopically broad flat scars of irregular distribution were taken as characteristic of pyelonephritic contraction; microscopically interstitial infiltration predominating in the medulla was regarded as supporting evidence.

On a histological basis the cases were divided into two main groups

<sup>\*</sup> From the Mallory Institute of Pathology, Boston City Hospital.

— acute and chronic. These were again subdivided into focal and diffuse lesions. Since the unilateral involvement of the kidneys could not possibly be causatively related to a general vascular hypertension, we planned these cases under the column of “focal” lesions for the special purpose of this investigation.

### ANALYSIS OF DATA

#### (1) *Occurrence of Hypertension and Renal Insufficiency in Pyelonephritis*

In acute focal pyelonephritis neither hypertension nor renal failure was encountered. Of 13 patients with acute diffuse pyelonephritis 9 died in uremia and hypertension was present in 4 of these. Two patients presented the picture of essential hypertension with superimposed diffuse acute pyelonephritis but without renal insufficiency.

In chronic pyelonephritis with focal or unilateral contraction of the kidney hypertension was present in 6 out of 9 cases and in 3 of these the individuals died in uremia. From the available evidence it appears that these 6 were cases of “primary” or “essential” hypertension with ischemic contraction complicated by focal pyelonephritis, the latter precipitating the uremic termination in 3 cases.

In chronic pyelonephritis involving the kidneys diffusely, hypertension and uremia were associated in 16 out of 26 cases; hypertension alone was present in 4 and uremia without hypertension in 6. In the majority of instances it is impossible to decide whether we are dealing with a primary “vascular” hypertension or with secondary “renal” hypertension. So far as our present investigation is concerned, however, such a differentiation is not essential.

#### (2) *Inflammatory Lesions in the Glomeruli in Pyelonephritis*

Ischemic changes in the glomeruli are very common and need no description. Inflammatory lesions, on the other hand, are comparatively rare. It is possible, however, to distinguish two types. The first resembles closely the lesion we have described as “alterative glomerulitis” in decompensated benign nephrosclerosis.<sup>1</sup> The second and more common type appears to be a direct extension of the inflammatory process from the interstitial tissue. Since this form of glomerulitis is given very little attention in the literature we describe



it in some detail. Accumulations of leukocytes and fibrin are frequently found in the periglomerular lymph spaces. In certain glomeruli these can be seen to break through Bowman's capsule into the capsular space (Figs. 1 and 2). The capsule itself may be invaded by leukocytes and its fibrous layer may undergo disintegration. The epithelial cells are lifted off the basement membrane, then degener-

TABLE I  
*Cases of Pyelonephritis Without Hypertension*

Autopsy No.	Age	Sex	N.P.N.	Diagnosis	Tubular dilatation	Alternative glomerulitis
32-330	58	M	31	Focal acute pyelonephritis	—	—
32-435	27	F		" " "	—	—
32-619	69	M	35	" " "	—	—
32-642	27	M	32	" " "	—	—
33-85	18	F	—	" " "	—	—
33-674	50	F	32	" " "	—	—
34-669	53	F	60	" " "	—	—
34-319	68	M	43	" " "	—	—
32-77	83	M	57	Diffuse acute pyelonephritis	Moderate	—
32-168	57	M	131	" " "	Moderate	—
32-238	72	M	—	" " "	Slight	—
32-264	40	M	33	" " "	—	—
32-337	44	F	39	" " "	—	—
33-448	77	M	55	" " "	Slight	—
34-417	55	M	70	" " "	—	—
32-494	21	F	60	Focal chronic pyelonephritis	Slight	—
33-583	48	M	27	" " "	Slight	—
34-446	57	M	33	" " "	Slight	—
32-384	74	M	95	Diffuse chronic pyelonephritis	—	—
33-378	53	F	167	" " "	Slight	—
34-138	61	M	100	" " "	Moderate	—
34-128	31	F	+	" " "	—	—
34-327	53	F	167	" " "	Slight	—
34-531	22	F	185	" " "	Severe	+

ate and desquamate. Leukocytes migrate into the capsular space and may be seen to encircle a normal looking glomerulus. Acute focal adhesions occur between the capsule and glomerular tuft. The exudate of fibrin and leukocytes can be seen to spread to the adjacent glomerular loop where it may remain localized or may extend to the rest of the glomerulus (Fig. 3). Adjacent to the point of capsular

TABLE II  
Cases of *Pylonephritis With Hypertension* \*

Autopsy No.	Age	Sex	Blood pressure	N.P.N.	Diagnosis	Tubular dilatation	Alternative glomerulitis
32-481	yrs. 69	M	100/90	53	Diffuse acute pyelonephritis	—	—
32-482	72	M	180/90	85	"	Considerable	—
33-304	72	M	(580 gm.)	54	"	Moderate	—
33-698	52	M	170/85	122	"	Considerable	+
34-92	41	M	250/160	135	"	Moderate	+
34-450	80	M	230/140	71	"	Severe	+
32-163	57	M	170/94	38	Focal chronic pyelonephritis	—	—
32-505	63	F	240/120	64	"	Considerable	+
32-656	54	F	240/120	120	"	Moderate	+
33-284	70	M	(460 gm.)	67	"	Considerable	—
33-528	53	M	208/100	48	"	Considerable	Only one glomerulus found
33-730	75	M	180/100	45	"	—	—
32-68	76	M	230/106	43	Diffuse chronic pyelonephritis	—	—
32-85	68	F	150/100	194	"	Severe	+
32-515	55	F	170/70	242	"	—	—
32-566	48	F	185/85	140	"	Slight	—
32-614	56	M	150/130	41	"	Moderate	—
32-626	62	F	200/90	195	"	Considerable	—
33-65	67	M	180/65	265	"	Considerable	—
33-397	70	M	(460 gm.)	67	"	Slight	—
33-690	78	M	(500 gm.)	122	"	Considerable	—
34-26	71	F	(570 gm.)	280	"	Considerable	+
34-101	39	M	170/110	250	"	Severe	—
34-695	66	M	(450 gm.)	89	"	Considerable	Only one glomerulus found
34-618	64	M	(500 gm.)	—	"	—	—
34-522	54	F	230/136	195	"	Considerable	Only one glomerulus found
34-502	32	M	180/110	200	"	Considerable	—
34-300	73	M	185/85	105	"	Considerable	—
32-368	55	M	200/94	57	"	Considerable	—
32-332	70	M	180/100	97	"	Slight	+
34-77	52	M	250/150	120	"	Severe	—
34-557	37	F		200	"	Severe	+

\* In some cases the blood pressure was not elevated on admission but a previous history of hypertension was present or an increased heart weight was recorded at autopsy (given in blood pressure column).

invasion proliferative glomerulitis, epithelial swelling and increase in leukocytes are occasionally seen confined to one glomerular loop (Fig. 4). In some glomeruli bland adhesions are present which may result from the above process. They can only be distinguished from the adhesions which follow "alterative" glomerulitis if the previous stages of their development can be recognized.

Alterative glomerulitis (Figs. 5 and 6) occurring in pyelonephritis is in no sense to be regarded as a direct extension of the inflammatory process from the interstitial tissue. The necrosis of the capillary loops originates in the glomerular tuft itself and leads to capsular adhesions from within. Capsular proliferation may be seen in both forms of glomerulitis but is a comparatively rare finding.

The distribution of these two types of glomerular lesions is characteristic. The "invasive" form is most common in areas of interstitial infiltration and may be found wherever such infiltration is present whether the process is unilateral or bilateral. The alterative type, on the other hand, may be found where interstitial infiltration is minimal or absent. We have seen this form of glomerulitis in a kidney which showed no other change than gross tubular dilatation in response to complete ascending atrophy of the opposite kidney. It is never present in a unilateral pyelonephritis where the remaining kidney shows no signs of functional impairment.

On comparing the histological and clinical data it appears that the invasive type of glomerulitis is in no way related to the occurrence of either hypertension or renal insufficiency. Alterative glomerulitis, on the other hand, bears a definite relation to these disturbances of function. In our series of 56 cases of pyelonephritis alterative glomerulitis occurred in 13. Twelve of these showed clinical evidence of hypertension and renal insufficiency. In the single exception death occurred in uremia but the blood pressure was normal (140/75 mm. Hg.), and at autopsy the heart weight was 240 gm. The clinical notes record the presence of retinal hemorrhages.

The nitrogen retention in these cases was of true renal origin, *i.e.* was associated clinically with impaired concentrating power of the urine and histologically with tubular dilatation.

## CONCLUSIONS

Two types of inflammatory lesions of focal distribution occur in the glomeruli in pyelonephritis. The first is peculiar to this condition and results from extension of the interstitial inflammation to the glomerulus. The second or "alterative" type of glomerulitis occurs in pyelonephritic contraction of the kidney as a manifestation of a generalized vascular disease. Clinically it is associated in the overwhelming majority of cases with hypertension and renal insufficiency. Histologically its distribution in the kidney is apparently independent of the interstitial inflammatory process. The lesion itself is indistinguishable from the focal glomerulitis which is found in essential hypertension of the "decompensated benign" or malignant types, in which also it is closely associated with renal insufficiency.

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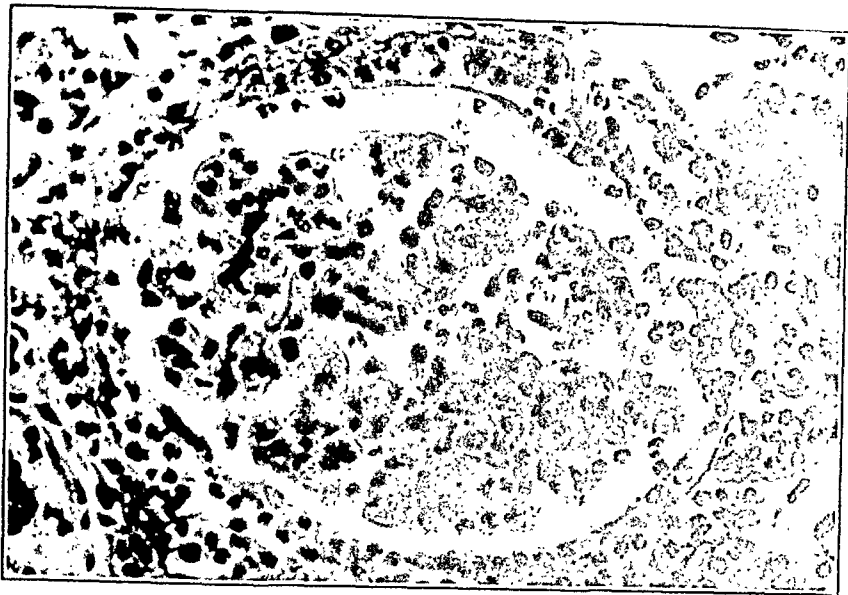
1. Kimmelstiel, P., and Wilson, C. Benign and malignant hypertension and nephrosclerosis. *Am. J. Path.*, 1936, 12, 45-81.
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4. Pfeiffer, A. Ueber die pyelonephritische Schrumpfnieren. *Ztschr. f. urol. Chir.*, 1933, 36, 53-91.

## DESCRIPTION OF PLATES

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### PLATE 13

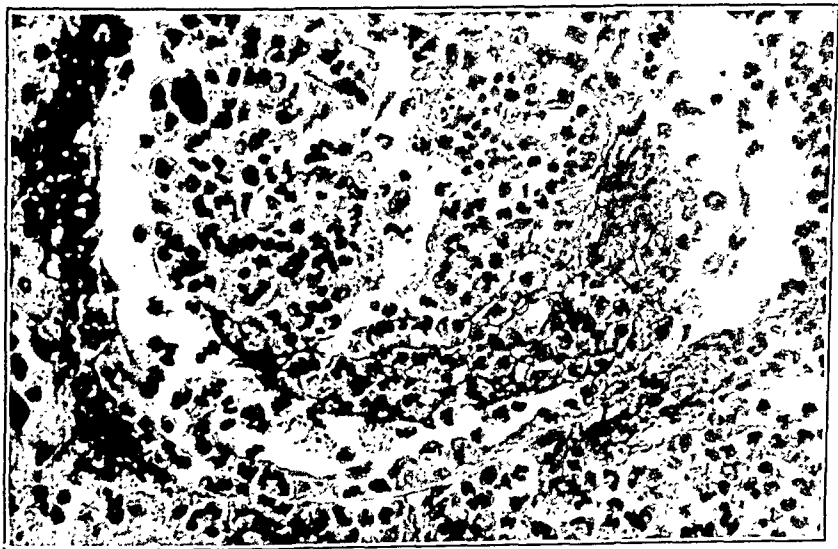
- FIG. 1. "Invasive" type of glomerulitis in pyelonephritis. Accumulation of leukocytes and fibrin in periglomerular lymph space invading Bowman's capsule. Eosin-methylene blue.
- FIG. 2. "Invasive" type of glomerulitis. Leukocytes invading Bowman's capsule at one point. Eosin-methylene blue.
- FIG. 3. "Invasive" type of glomerulitis; acute adhesions with Bowman's capsule at point where exudate of fibrin and leukocytes invades Bowman's capsule and part of glomerular tuft. Eosin-methylene blue.



I

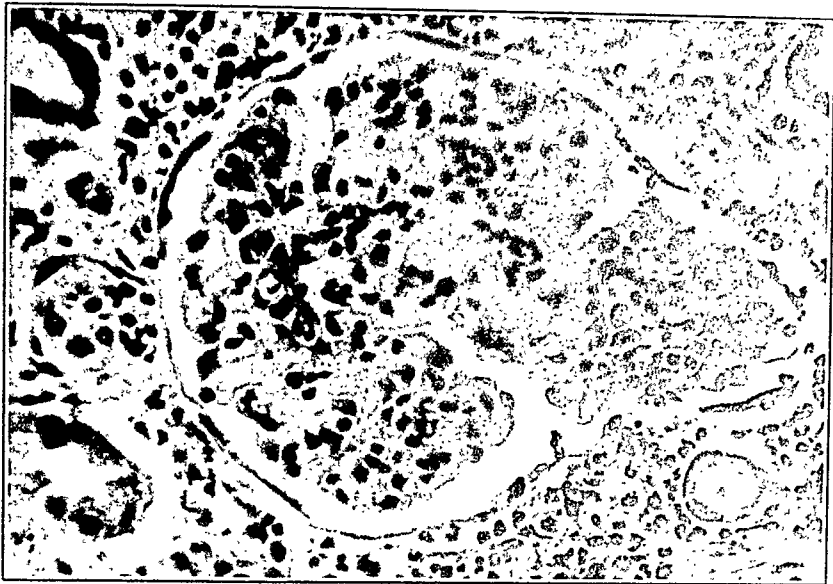


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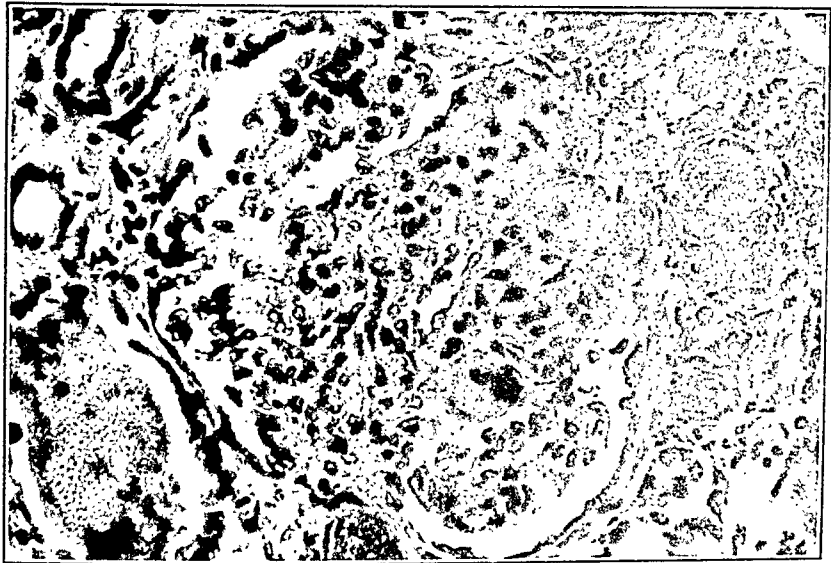


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- FIG. 4. "Invasive" glomerulonephritis. Epithelial swelling and increase of nuclei in outer part of glomerular tuft adjacent to point of invasion. Eosin-methylene blue.
- FIG. 5. Altered course of glomerulonephritis. Adhesive glomerulitis with hyaline cast in one large vessel, all active necrosis in some others. Eosin-methylene blue.
- FIG. 6. Altered glomerulitis in pyelonephritis. Adhesion with early degenerative changes in adjacent glomerular tuft. Eosin-methylene blue.



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## A STUDY OF ADRENAL CORTEX MORPHOLOGY \*

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In our experimental studies on the physiology of the adrenal gland we looked for morphological criteria whereby we could determine the degree of activity and the state of exhaustion of the adrenal cortex.† The accepted methods did not entirely meet critical analysis. Variations in the amount and distribution of lipoids seemed to depend too much upon the technique of fixation and the care used in embedding and sectioning. The size and weight of the gland also proved to be of limited use in determining its functional state.

### MATERIALS AND METHODS

The adrenal glands of normal frogs, mice, rats, guinea pigs, rabbits, cats, dogs, monkeys and humans ‡ have been studied as reference controls. These form the basis for a determination of the changes taking place under abnormal conditions. The latter have been experimentally produced and also observed in naturally occurring infections. A variety of experimental procedures was undertaken in an effort to produce specific cortico-adrenal lesions. Among the substances used were bacterial toxins, thyroxin, parathormone, adrenalin, histamine, mercuric chloride, and extensive body burns were also employed.

Although our own experimental material was prepared for microscopic examination by a variety of special techniques, the majority of adrenal glands which others have given us the opportunity to study were fixed in ordinary fixatives and stained with hematoxylin and eosin. A scheme of identification was gradually developed and tested with many series of "unknown" glands.

\* Received for publication July 26, 1935.

† A preliminary paper was read before the American Association of Anatomists, March 1934. *Anat. Record*, 1934, 58, 43 (No. 4 and Suppl.).

‡ A human adrenal gland that is normal by our standards is very difficult to obtain.

## OBSERVATIONS

Before undertaking a detailed description of the morphological changes accompanying certain physiological and pathological states we shall mention briefly our conception of the life history of the adrenal cortex cell. This is discussed in detail in a paper by Zwemer, Wotton, Nussman and Norkus.<sup>1</sup> We agree with Mulon,<sup>2</sup> Bogomolez,<sup>3</sup> Goormaghtigh,<sup>4</sup> and Hoerr<sup>5</sup> that the adrenal cortex grows from without inward, new cells being formed at the periphery and destroyed in the reticular zone near the medulla. That the glomerular cells arise from indifferent connective tissue-like cells in the capsule is our additional contribution. These capsular cells lose their long processes, become short ovals and take up lipid droplets. A further increase in the amount of cytoplasm and a marked increase in cell fats mark the transition to the spongiocytes. The latter retain and emulsify their fat content as they are gradually pushed inward by the formation of new cells. After an experimentally induced discharge from these cells, lipid can be demonstrated in the blood stream in the form of small fat droplets by a special gelatin embedding lipid technique (Zwemer<sup>6</sup>). Direct observation of living frog adrenal glands, under normal and experimental conditions (Singer and Zwemer<sup>7</sup>), strengthens the view that lipoids may be extruded from the cells in droplet form as well as in non-visible secretions.

As the cells secrete, the ratio of cytoplasm to nucleus is greatly diminished, so that the innermost regions of the cell cords consist of rows of nuclei with very small remnants of cytoplasm still surrounding them. In the end stage the cell is represented by a pyknotic nucleus which is finally phagocytozed.

These studies show that the fundamental plan of adrenal cortex cell progression is the same for all species investigated. The differences between normal glands of various species appear to be due to changes in the relative proportions of the cell types. For ease in description we shall limit the detailed discussion to adult mammalian adrenal glands, with particular reference to those of carnivores and primates. The picture, as seen in a segment of a median section of the gland, will be described and illustrated.

A normal gland (Fig. 1) shows the capsule, a single or incomplete double row of glomerular loops, a moderately wide fascicular zone composed of spongiocytes in the outer half and smaller cells in vari-

ous states of discharge in the inner half. Internal to this region and near the medulla is the network of cell cords known as the "reticular zone." In its wide capillary meshes are found many macrophages; these assist in removing the cellular débris of exhausted and incompletely discharged cells.

The first modification of the normal picture reveals an unusually large number of spongiocytes present in the entire fascicular zone. The reticular zone is reduced to a small band of cell cords. This type of gland we have called the lipoid storage type (Fig. 2). A minor modification may show the capsule to be thickened and an increased number of circles and loops of cells is present in the glomerular zone. This latter picture is due to stimulation of the cortico-adrenal cells to hypertrophy and hyperplasia. It may occur as a result of anterior pituitary, mild thyroid, or other treatment.

With an acute demand for cortico-adrenal secretion (Fig. 3) there is a rapid change in the reticular and inner fascicular cords. The outer zones apparently remain unchanged and the adrenal cortex in some cases of death in the acute stage gives the appearance of having an ample store of cortical lipoids. In spite of this apparent store the bodily condition and blood chemistry may indicate a state of adrenal insufficiency and a careful study of sections stained for lipoid shows that the visible fat present is in much larger droplets and often stains differently from normal. This lipoid retention with insufficiency is perhaps a result of the time factor. If we suppose the existence of a polymer of cortin or a precortin, time would be necessary for this substance to be converted into the active hormone. In an acute demand adrenal insufficiency may appear before this change can take place. A very acute demand results in an exhausted gland with little indication of new cell formation (Fig. 4).

When the demand is mild and has existed for only a short period of time, the adrenal morphology is also different (Fig. 5). The glomerular zone is wider, but composed of somewhat smaller cells, spongiocytes have almost disappeared, and the inner fascicular zone is extensive with narrow irregular columns of small cells. The reticular zone does not show the accumulation of débris that is found with acute demand. If the mild demand has occurred for some time the hyperplasia may result in an adrenal that is larger than normal. The cell columns are very narrow and the larger capillaries may be congested (Fig. 6). In spite of an enlarged gland, the animal may suffer

from adrenal insufficiency. One can have a large depleted adrenal in addition to having a large, well stored gland.

A severe but not necessarily acute exhaustion of the adrenal cortex modifies the foregoing picture (Fig. 7). The capsule is thickened and in it accessory or "adenomatous" masses may be embedded. The glomerular zone is wide, quite irregular, and frequently has layers of cells which are crescentic in cross-section, the convexities being directed inward, as found by Hoerr.<sup>5</sup> This directional curve can be seen best in adrenal glands that have been actively stimulated. The fascicular zone is irregular and is composed of small cells having a diminished amount of cytoplasm. The cell columns frequently are widely separated by large capillaries. The reticular debris accumulates faster than it can be removed by macrophages. In this type we see that in spite of the rapid formation of new cells there is little accumulation of lipoid (and its associated hormone). The life history of these small cells is probably extremely short, and an individual with this type of gland can easily be thrown into complete adrenal insufficiency by a slight additional demand for the vital secretion. A small accessory gland that is maintaining life in an experimental animal often has this appearance.

If a period of severe prolonged demand continues until death occurs from uncomplicated adrenal insufficiency, we see an exhausted adrenal (Fig. 8). This type of gland has no definite fascicular zone and there is a rapid transition from the accumulative to the degenerating phases. New cell formation is not sufficiently rapid to keep up with the secretory or discharge phase, and no storage is apparent.\*

### DISCUSSION

On the basis of our observations we have attempted to describe eight conditions present in adult mammalian adrenal glands. There

\* A series of "unknown" adrenals from 200 human autopsies was very kindly put at our disposal by Dr. Seecof and checked by him from his and our records. A comparison of our classification with the pathologists' findings showed a close correlation of adrenal cortex morphology with the functional demands one might expect from the cause of death. In 80 per cent of the cases the group to which the gland belonged was easy to determine; the remaining 20 per cent of the cases were difficult to determine for two reasons. When the adrenals were from very young children the organization of the gland was incomplete. The sections frequently showed rapid multiplication of cells in the capsule and outer glomerular zone and there were also a number of so-called adenomas of cortical tissue. In young adrenals this appearance is due to portions of cortex that have failed as yet to become incorporated within the gland capsule. Another difficult group consisted of adrenals from cases of unusual causes of death.

are, however, gradations between these, and additional complicated types resulting from experimental procedures. An example of complication would be a gland that had been stimulated to hyperplasia and hypertrophy and then subjected to an acute demand.

The changes occurring in the adrenal cortex cells during normal metabolism seem to be accelerated by increased catabolism in other parts of the body.

To determine adequately the condition of a gland under consideration one must, of course, first know the normal proportions of the formative, loaded and discharging types of cells for the species studied. For example, in some species the normal gland seems at first to be composed entirely of loaded spongiocytes and the typical cell sequence becomes more apparent only after experimental procedure. For these species (frog, turtle) the storage type of gland seems to be the normal condition for certain seasons of the year. In other species, particularly among the mammals, there is a more rapid cell utilization, and cells in all stages can be found in a single segment of a median section. Among mammals there is a species difference in the amount of spongiocyte tissue normally found. This has been noted by others and expressed as the amount of visible lipid found in the outer fascicular zone.

It should be noted that the cell columns in different segments of a single section may vary considerably as a result of intermittent blood circulation. An estimation of the state of the gland as a whole depends, of course, on the condition present in most of the sections. If one wishes to determine minor changes, some commonly used quantitative measurement of relative proportions of cell types may be employed. This may be by cell counts, by polarplanimetric measurement of different cell type areas, or by weighing paper outlines of areas.

One of the fundamental features of the normal adrenal cortex in all types studied is the large cell containing much visible lipid in a finely divided state. The lipoids are undoubtedly there for some purpose. This point needs no emphasis since the functional state of the gland has long been gauged by its lipid content. Furthermore, chemical studies have shown that the vital hormone, although somewhat water soluble, is extracted with the lipoids by organic solvents. One can conceive the spongiocyte lipid to be either the raw substance from which the hormone is made, or the vehicle in which it is

stored. Perhaps both functions are performed by the lipoids. If the hormone were not stored in a protected and relatively inactive form, it might well affect the structure of the cells by which it is formed (*cf.* gastric secretions and postmortem autolysis). By conceding that the spongiocyte is the cortico-adrenal lipoid storage cell, one admits the presence of accumulating and discharging cells. The rapidity with which new cells are formed is one factor in an increased gland size, but the rapid hypertrophy of these new cells as a result of their lipoid storage also contributes to the increase. If lipoids are excessively stored the gland size increases quite rapidly. On the other hand, if the discharging phase predominates, one could expect to find a small gland with very few of the loaded, spongiocyte cells. Rapid cell proliferation, however, might give rise to a large gland. As in the thyroid gland, an increased activity results in a different morphological appearance from that seen in storage, although in both cases the glands may be enlarged.

This broad plan of viewing adrenal cortex changes taking place under natural conditions was applied to specific lesions induced by bacterial and other toxins. In these cases the adrenal gland morphology depended on the extent of the infection, the amount of toxin injected or produced, and the rapidity with which death occurred, *i.e.* the length of time over which the toxin had acted.

No attempt has been made to discuss fully the pathology of the adrenal glands. This has been extensively reviewed by Goldzieher.<sup>8</sup>

#### SUMMARY AND CONCLUSIONS

The morphology of the adrenal cortex seems to reflect the demands imposed by body needs.

An excess of hormone over normal requirements is shown by an increase in the number of storage cells.

An acute demand discharges the mature cells but does not immediately affect the accumulative cell types.

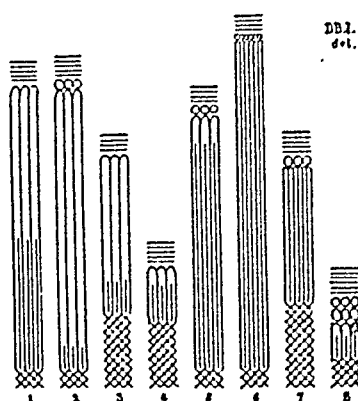
Prolonged demands stimulate new cell formation, which may or may not be able to counteract the cell utilization of the discharging phase.

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## DIAGRAM OF CORTICO-ADRENAL TYPES



The accompanying schematic diagram may aid in an understanding of the plate by indicating the relative proportions of cell types generally present in the different morphological states of the adrenal cortex.

*Horizontal lines* at the top of a figure indicate the capsule.

*Circles and arches* are used for the small proliferating new cells (zona glomerulosa).

*Vertical lines* represent the columns of cells bounded by capillaries (zona fasciculata).

(a) If the lines are widely spaced the cells are large and loaded with lipid droplets (spongiocytes).

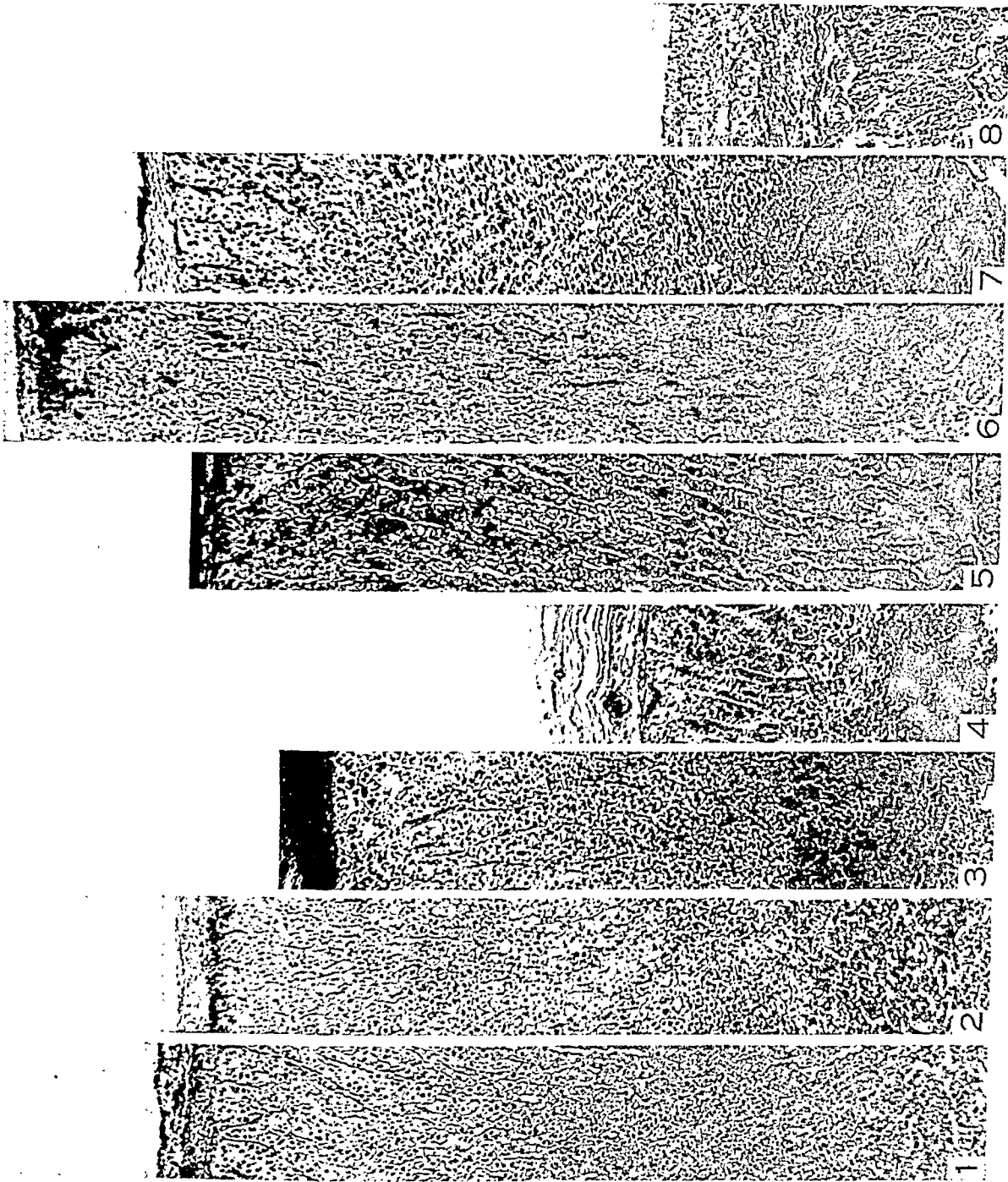
(b) If the lines are close together the cells are of the active secreting type.

*Cross-hatched areas* correspond to the inner network of cell cords with its sinusoids, macrophages and cellular debris (zona reticularis).

## DESCRIPTION OF PLATE

## PLATE 15

- FIG. 1. *Normal* secreting adrenal cortex with a balance between accumulating and discharging cell types.
- FIG. 2. *Lipoid storage* with the accumulative cells predominating.
- FIG. 3. An *acute demand* results in a rapid cytoplasmic diminution in the inner zones.
- FIG. 4. A *severe acute* demand depletes the gland with little or no indication of new cell formation.
- FIG. 5. A *mild stimulation* to discharge is indicated by a decrease in the number of lipid-loaded spongiocytes.
- FIG. 6. *Prolonged activity* is associated with an increase in accumulative and discharging cell types and an absence of lipid storage cells.
- FIG. 7. In a *severe* condition, new cell formation and discharge follow each other rapidly and the normal, regularly arranged cell columns no longer are present.
- FIG. 8. *Severe prolongation* of the demand results in an atrophic adrenal with groups of cells separated by fibrous connective tissue which no longer resembles the reticular tissue usually present.





# A CHEMICAL ANALYSIS OF ATHEROSCLEROTIC LESIONS IN HUMAN AORTAS \*

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The few chemical studies of atherosclerotic aortas which were reported before 1933 are well summarized in Cowdry's<sup>1</sup> survey of arteriosclerosis. In August, 1934, Meeker and Jobling<sup>2</sup> reported analyses for lipids on a series of aortas. In October, 1934, Rosenthal<sup>3</sup> reported analyses for total fat content in aortas and based certain conclusions on the assumption that "the proportions of the fatty constituents remain constant in atherosclerosis." During the spring of 1934 analyses of aortas for lipids were made in this laboratory in connection with a report on atherosclerosis which was being prepared for the annual meeting of the American Heart Association. The results of these analyses are strikingly similar to those of Meeker and Jobling, and do not substantiate Rosenthal's "constant lipid proportion" hypothesis. Also, they do not confirm the earlier work of Schönheimer<sup>4</sup> who found a steadily increasing ratio of ester to free cholesterol with advancing atherosclerosis.

The tissue analyzed in this laboratory consisted of sections of aortas from 11 adults. Since arteriosclerosis is a patchy lesion and may vary in a given aorta from very slight atheroma to far advanced lesions with varying degrees of fibrosis, calcification, ulceration and thrombosis, it seemed far more valuable to analyze individual lesions than entire aortas, as had been done by many earlier investigators. Also, since lesions which differ considerably microscopically often appear very similar to the naked eye, each lesion analyzed was sampled for microscopic study. In order to control to some degree the factors of age and individual variation, several sections were taken from each aorta and included relatively normal appearing areas as well as atherosclerotic lesions.

The sections were obtained at autopsy, care being taken to prevent contamination with any fat-containing substance. The pieces of tissue were washed quickly to remove any clotted blood and blotted

\* Received for publication August 2, 1935.

dry with fat-free filter paper. The adventitia was then stripped off in order to remove the adventitial fat. After a little practice it was found possible to make the line of cleavage occur at relatively the same plane in the wall of each aorta. This was checked microscopically. From these adventitia-free portions of aorta several samples were dissected, care being taken that all parts of a given sample should appear uniform. From each sample a representative slice was placed in 10 per cent neutral formalin for microscopic study. From the remainder of each sample several half gram portions were weighed to 1 mg. accuracy and each portion was stored in a small amount of alcohol-ether mixture.

The chemical methods used were essentially those of Bloor<sup>5</sup> and Yasuda,<sup>6</sup> with certain minor modifications because of the tissue used or the equipment available. The lipids were extracted by grinding the sample of aorta in a mortar with a small portion of fat-free ground glass, a few cubic centimeters of distilled water being added to make a paste. Then a small amount of alcohol-ether mixture was added and the grinding continued. From time to time the solute was decanted into a fat-free filter, more solution added to the mortar, and the grinding continued, using successively several small portions of cold alcohol-ether mixture, hot alcohol-ether, ether, and lastly, boiling absolute alcohol. The filtrate at this point was often slightly cloudy, but refiltration produced a clear solution, even though the filter was repeatedly washed with small portions of the same solutions as those used in the first filtration. The filters were saved and the residues from several cases re-extracted together and the filtrates analyzed to make sure that practically all of the lipids were extracted each time.

The final filtrates were collected in 100 cc. volumetric flasks, which were then filled to the mark and aliquots taken for analysis.

Yasuda's modification of Bloor's method for free and total cholesterol was used. It consisted essentially in precipitating the cholesterol with digitonin, freeing the digitonide from other lipids and from excess digitonin, dissolving the isolated digitonide in boiling hot alcohol, evaporating to absence of alcohol, oxidation of the residue with 1 N potassium dichromate, using Nicloux' solution as a catalyst, and finally titrating the excess dichromate with 0.1 N sodium thiosulphate. When determining total cholesterol, the samples were saponified with sodium ethylate before precipitation with

digitonin. The figures for cholesterol esters were obtained by subtracting those for free cholesterol from total cholesterol. Since 10.62 cc. of 0.1 N potassium dichromate are needed to oxidize 1 mg. of cholesterol as digitonide,

$$\frac{\text{cc. of 0.1 N K}_2\text{CrO}_7 \text{ used}}{10.62} = \text{mg. cholesterol in aliquot analyzed.}$$

Lecithin was determined by precipitating it with acetone and magnesium chloride, isolating the precipitate and dissolving it in moist ether. The solute was then evaporated and the residue oxidized as for cholesterol. No attempt was made to separate lecithin from the other phospholipids, so the percentages given probably include some cephalin.

The figures for total lipid were obtained by oxidizing together the total fatty acids and total cholesterol in aliquots of the alcohol-ether extract which were saponified, acidified, and extracted with petroleum ether. By subtracting the amount of potassium dichromate used to oxidize the total cholesterol from that used for total lipid the figures for fatty acids were obtained. The latter are not wholly reliable since some of the more volatile fatty acids probably were lost when the petroleum ether extracts were evaporated. There are also other imperfections in the above procedures, but these methods were chosen for this investigation because, in this laboratory, the recovery of lipids from known test samples by these methods has approached more nearly and consistently the theoretical values than when other methods have been used.

The types of cases from which sections of aorta were taken are included in Table I.

In Table II the results of gross, microscopic and chemical studies on the various sections of aortas are listed by cases. The sections from the more and from the less atheromatous areas of the same aorta are identified by the use of a letter following the case number. In all instances two or more samples were taken from each area (A, B, C, D, and so on) of each aorta for analysis. When these duplicate samples differed 0.1 per cent or more the extremes are given.

In each of 6 cases there was a marked difference in the degree of atherosclerosis in the two or more types of samples taken, and in each instance the more atheromatous portions yielded higher percentages of each lipid than the more normal portions of the same

TABLE I  
*Types of Cases Analyzed*

Case No.	Age	Sex	Hours post-mortem	Cause of death	Other findings
1	yrs. 54	M	3	Lobar pneumonia	Extensive cellulitis, probable septicemia
2	53	M	6	Lobar pneumonia	Advanced general arteriosclerosis with cerebral degeneration
3	43	F	4	Confluent lobular pneumonia	Bronchiectasis, chronic biliary disease, general arteriosclerosis, chronic pyelitis, vestigial right kidney
4	62	F	14	General arteriosclerosis with cerebral softening	Coronary sclerosis with myocardial degeneration
5	54	M	5	Urethral stricture with pyelonephritis	Generalized arteriosclerosis
6	29	F	6	Confluent lobular pneumonia	Tubo-ovarian abscess with pelvic peritonitis, arrested pulmonary tuberculosis, left leg amputated in childhood
7	47	F	6	Intestinal obstruction	Toxic adenoma of thyroid, healed hysterectomy wound
8	50	F	9	Lobular pneumonia	Cirrhosis of liver, exophthalmic goiter with thyroid heart disease, healed panhysterectomy wound
9	75	M	4	Lobular pneumonia with empyema	Bronchiectasis, adenoma of the prostate
10	74	M	5	Lobular pneumonia	Epidermoid carcinoma of bladder, chronic biliary disease
11	50	M	6?	Gunshot wound with severance of common carotid artery and fatal hemorrhage	Essentially normal

TABLE II  
Morphological and Chemical Findings

Case No.	Area	Gross plaques	Ulceration	Calcification	Lipoid cells	Pooling of lipids	Increased connective tissue	Thinning of media	Inflammation	Lipoid crystals	Total cholesterol	Free cholesterol	Cholesterol esters	Fatty acids	Lecithin	Total lipid *
1	C E	o +	o o	o o	+ +	o + + +	+ + + +	o + +	+ +	o +	per cent 1.0	per cent 0.7	per cent 0.3	per cent 2.3- 3.6	per cent	0.12-0.17 0.53-0.61
											2.0-2.6	0.7-1.2	1.3-1.4	12.7-14.2		
2	B A	o + +	o o	o +	+ +	o + + +	+ + + +	o + +	o +	o +	1.7-1.8 2.6-3.3	1.5 1.7	0.2 0.9-1.6	4.4	0.5 0.9	0.13-0.22
3	B C A	o + + +	o o +	o o +	+ + +	o + + +	+ + + +	+ + + +	o + + +	o + + +	1.0-1.4 2.2-2.9 3.6-4.1	0.3-0.4 1.0-1.2 2.1-2.2	0.6-1.2 1.1-1.7 1.5-2.0	2.7-4.0 2.1-3.2 5.6-7.4	0.7 0.7 1.8	0.14-0.20 0.19-0.20 0.34-0.43
4	B A	o +	o o	o +	o +	o +	o + +	o +	o o	o o	0.4-0.6 2.0-2.3	0.3 0.9-1.1	0.1-0.3 1.0-1.3	0.7-0.9 5.0-5.1		0.04-0.06 0.26-0.27
5	B A	o + +	o o	o +	+ +	o + + +	+ + + +	o + +	o +	o + +	0.5-0.6 2.5-3.4	0.3-0.4 1.6	0.2 0.9-1.8	0.2-0.6 2.1-3.1	0.2-0.6 0.9-1.2	0.03-0.05 0.21
6	B A	o + + +	o o	o +	+ +	o + + +	+ + + +	o + +	o + + +	o + +	0.4-0.5 2.4	0.3 1.8-1.9	0.1-0.2 0.5-0.6	1.6-1.8 6.7-7.9	0.2-0.3 0.5-0.7	0.07-0.09 0.34
7	A	+	o	o	+	+	+	+	o	o	1.8-1.9	0.4-0.7	1.1-1.5	7.0		0.33
8	A B	o o	o o	o o	+ +	o o	+ +	o o	o o	o o	1.4 1.8	1.0-1.1 1.5	0.3-0.4 0.3	5.1 2.9-6.4	0.4 0.6-1.0	0.24 0.17-0.30
9	B A	o + +	o o	o +	+ +	o + + +	+ + + +	o + +	+ +	o + +	1.6-1.7 2.1-2.3	0.4 0.7-0.8	1.2-1.3 1.4-1.5	0.4-0.8 5.4-6.5	1.4-1.8 1.4-1.8	0.08-0.09 0.28-0.33
10	A	+	+	o	+	+	+	+	+	+	5.5	3.6-4.1	1.4-1.9	16.1-16.7		0.64-0.89
11	A B	o +	o o	o o	+ +	o +	+ +	o o	o o	o o	1.2-1.5 1.6-1.9	0.3 0.6	0.9-1.2 1.0-1.3	3.1-3.4 4.1-4.5		0.17 0.22

\* cc. 0.1 N K<sub>2</sub>CrO<sub>7</sub> required to oxidize lipids from 1 mg. aorta.



aorta. In 2 other cases (Nos. 3 and 9) this was also true, with the exception of lecithin and fatty acids in the one case, and lecithin in the other. In 2 cases (Nos. 7 and 10) analyses were completed on only the more atheromatous samples. In case No. 8 the types of samples taken appeared to be similar morphologically but were from widely separated areas of aorta; no significant difference in the amount of lipids was found in these samples.

Therefore, it may be concluded that portions of a given aorta which appear alike microscopically are also similar chemically in so far as their lipid content is concerned, and that areas presenting more lipids microscopically contain more lipids chemically. These facts indicate that the atheromatous process in the aorta is essentially different from so-called fatty "degeneration" in such organs as the heart and kidneys where lipids may seem to be increased, as determined by microscopic methods, but are the same in amount, or even decreased, chemically.

In Table III the samples of aortas are arranged according to the degree of atherosclerosis morphologically, without regard to cases. Considering both morphological and chemical data, the samples of aortas seem to fall into five main groups:

Group I. A section of aorta from a female 62 years of age, which showed no evidence of atherosclerosis either grossly or microscopically (Fig. 1).

Group II. Eight sections of aortas in which the lesions consisted of infiltration of the intima with lipid cells, and not more than a moderate increase in connective tissue. In these sections there were no gross plaques, no ulceration or calcification, no breaking of lipid cells with pooling of lipids, and no discernible lipid crystals. In only one section was there microscopic evidence of thinning of the media and this was accompanied by a moderate increase in intimal connective tissue (Fig. 2).

Group III. Four sections in which there was beginning pooling of lipids in the intima. In three of these the gross lesion was a plaque. In the one showing no plaque the fatty acid content was unusually low, considering the type of lesion. In these sections thinning of the media was again found in those areas which presented the greatest increase of intimal connective tissue (Fig. 3).

Group IV. Six sections of gross plaques, four of them calcified but none ulcerated, which microscopically showed marked pooling

of lipids and increased intimal connective tissue. In all of them lipid crystals were found and in all there was some inflammatory reaction (Fig. 4).

Group V. Two sections of plaques similar to those in Group IV, but with the addition of ulceration and much more numerous lipid crystals (Fig. 5).

All of the figures for lipids in Table III, except those for total lipid, indicate per cent of wet weight of aorta. Since the proportion of each lipid substance represented in the "total lipid" is not known, the oxidation value for the latter cannot be reduced to percentage. Therefore, the figures in Table III for total lipid indicate cubic centimeters of 0.1 N potassium dichromate used to oxidize all of the lipids extracted from 1 mg. of aorta.

In order to be able to compare more readily the percentages of lipids in the various types of lesions, the mean and its standard deviation for each lipid in each of the five groups were calculated. As values for the frequencies, all determinations on all aortas in the group were used, in calculating the mean for that group. The mean for each group was also calculated by using the average percentage for each aorta as the value of each frequency. The results of the two methods of calculation were so similar that only one set of figures is given (Table IV).

Meeker and Jobling,<sup>2</sup> when reporting their analyses, did not list their results for each lipid as per cent of wet weight of aorta, but such percentages are readily obtainable from their figures by multiplying lipid per cent of fatty extract by extract per cent of wet tissue. This was done and the mean for each lipid was calculated. Table IV shows the close similarity of results obtained in the two laboratories.

The differences in terminology are readily explained by the fact that the diagnoses of Meeker and Jobling were based on gross examination, while our terms refer to microscopic morphology. Thus, the lesions which we designate as "slight atherosclerosis" appeared normal in the gross, except for slight yellowish discoloration. The "early lesions" of Meeker and Jobling were described by them as being "small, raised, yellowish, opaque, glistening plaques," which description corresponds to the gross appearance of our "moderate atherosclerosis."

Although the variations in the percentages within the groups, as obtained in both laboratories, are too large to attach exact mathe-

TABLE III

## Degree of Atherosclerosis

Case No.	Area	Gross plaques	Ulceration	Calcification	Lipid cells	Pooring of lipids	Increased connective tissue	Thinning of media	Inflammatory reaction	Lipoid crystals	Total cholesterol	Free cholesterol	Cholesterol esters	Fatty acids	lecithin	Total lipid *	Age	Sex	Group
4	B	o	o	o	o	o	o	o	o	o	per cent 0.4-0.6	per cent 0.3	per cent 0.1-0.3	per cent 0.7-0.9	per cent	0.04-0.06	yrs. 62	F	I
6	B	o	o	o	+	+	+	o	o	o	0.4-0.5	0.3	0.1-0.2	1.6-1.8	0.2-0.3	0.07-0.09	29	F	II
5	B	o	o	o	+	+	+	o	o	o	0.5-0.6	0.3-0.4	0.2	0.2-0.6	0.2-0.6	0.03-0.05	54	M	
1	C	o	o	o	+	+	+	o	+	o	1.0	0.7	0.3	2.3-3.6		0.12-0.17	54	M	
11	A	o	o	o	+	+	+	o	o	o	1.2-1.5	0.3	0.9-1.2	3.1-3.4		0.17	50	M	
8	A	o	o	o	+	+	+	o	o	o	1.4	1.0-1.1	0.3-0.4	5.1	0.4	0.24	50	F	
3	B	o	o	o	+	+	+	o	o	o	1.0-1.4	0.3-0.4	0.6-1.2	2.7-4.0	0.7	0.14-0.20	43	F	
2	B	o	o	o	+	+	+	o	o	o	1.7-1.8	1.5	0.2	4.4	0.5	0.13-0.22	53	M	
8	B	o	o	o	+	+	+	o	o	o	1.8	1.5	0.3	2.9-6.4	0.6-1.0	0.17-0.30	50	F	
9	B	o	o	o	+	+	+	o	+	o	1.6-1.7	0.4	1.2-1.3	0.4-0.8	1.4-1.8	0.08-0.09	75	M	III
11	B	+	o	o	+	+	+	o	+	o	1.6-1.9	0.6	1.0-1.3	4.1-4.5		0.22	50	M	
7	A	+	o	o	+	+	+	+	o	o	1.8-1.9	0.4-0.7	1.1-1.5	7.0		0.33	47	F	
4	A	+	o	+	+	+	+	+	o	o	2.0-2.3	0.9-1.1	1.0-1.3	5.0-5.1		0.26-0.27	62	F	
9	A	++	o	+	+	+	+	+	+	++	2.1-2.3	0.7-0.8	1.4-1.5	5.4-6.5	1.4-1.8	0.28-0.33	75	M	IV
1	E	+	o	o	+	+	+	+	+	+	2.0-2.6	0.7-1.2	1.3-1.4	12.7-14.2		0.53-0.61	54	M	
3	C	++	o	o	+	+	+	+	+	+	2.2-2.9	1.0-1.2	1.1-1.7	2.1-3.2	0.7	0.19-0.20	43	F	
5	A	++	o	+	+	+	+	+	+	+	2.5-3.4	1.6	0.9-1.8	2.1-3.1	0.9-1.2	0.21	54	M	
2	A	++	o	+	+	+	+	+	+	+	2.6-3.3	1.7	0.9-1.6		0.9		53	M	
6	A	+++	o	+	+	+	+	+	+	+	2.4	1.8-1.9	0.5-0.6	6.7-7.9	0.5-0.7	0.34	29	F	
3	A	+++	+	+	+	+	+	+	+	+++	3.6-4.1	2.1-2.2	1.5-2.0	5.6-7.4	1.8	0.34-0.43	43	F	V
10	A	+	+	+	+	+	+	+	+	+++	5.5	3.6-4.1	1.4-1.9	16.1-16.7		0.64-0.89	74	M	

\* Expressed as cc. 0.1 NK<sub>2</sub>CrO<sub>7</sub> required to oxidize lipids from 1 mg. aorta.

TABLE IV  
*Mean Percentages of Wet Weight of Aortas*

Tissue	Total cholesterol		Free cholesterol		Cholesterol esters		Fatty acids	Phospholipids		Ratio of free to ester cholesterol	
	Z.†	M. & J.†	Z.	M. & J.	Z.	M. & J.	Z.	Z.	M. & J.	Z.	M. & J.
No atherosclerosis .....	0.5	0.4	0.3	0.2	0.2	0.2	1.0		0.3	1.5	1.0
Slight atherosclerosis .....	1.2		0.7		0.5		3.0	0.6		1.4	
Moderate atherosclerosis (grossly, early lesions *) .....	1.9	2.1	0.7	0.7	1.3	1.4	4.9	1.6	0.9	0.54	0.56
Marked atherosclerosis (grossly, medium lesions *) .....	2.6	3.4	1.3	1.3	1.3	2.0	6.5	1.1	1.2	1.0	0.73
Far advanced atherosclerosis with ulceration (grossly, late lesions *)	4.8	4.3	3.0	2.1	1.7	2.1	11.5	1.8	1.4	1.8	1.04

\* The gross diagnoses are those used by Meeker and Jobling.

† The initials refer to the authors Zeek,<sup>7</sup> and Meeker and Jobling<sup>2</sup> respectively.

mathematical significance to the calculated means, yet certain definite trends are shown by these figures, which add a little to our very meager knowledge of the process arteriosclerosis. (1) The percentages of total cholesterol and fatty acids vary directly with the severity of the lesions. (2) The chemical increase in total cholesterol and fatty acids begins with the earliest departures from the normal morphology. The accumulation of lipids in the intima does not represent a secondary regressive change in a fibrotic intimal plaque which has become too large for its blood supply, as has often been asserted, because the chemical increase in lipids begins before there is any plaque morphologically. (3) There is no assurance that all of the additional lipids found chemically in these lesions are visible microscopically as lipids. It is a well known fact that lipids may be increased or decreased chemically in lesions without a corresponding change morphologically. However, if it is assumed that the additional chemical lipids are contained in the added morphological elements it will be noted that the cholesterol of the infiltrating lipoid cells and loose connective tissue must occur chiefly as cholesterol esters, since the increase in cholesterol esters during the early stages of the lesions is much more marked than the increase in free cholesterol. However, when large pools of lipids form in the intima, and especially when these pools ulcerate into the lumen and are no longer sealed off from the lumen blood stream, the increase in free cholesterol becomes predominant and the ratio of free to ester cholesterol rises until it equals or exceeds that of adult normal aortic tissue. This increase in the proportion of free cholesterol in late lesions has been stressed by Meeker and Jobling, but is contrary to the findings of Schönheimer.

This investigation has not revealed the source of the lipids which appear in atheromatous aortas; however, the changing cholesterol ratios point definitely to some process other than simple imbibition from the lumen blood stream. Against this too long unquestioned hypothesis there also is accumulating evidence which arouses doubts as to the rôle which high blood lipids can play in causing atherosclerosis. Examples of such evidence come from autopsies on cases of lipoid nephrosis in which there is often extreme hypercholesterolemia without marked atherosclerosis. The deposition of cholesterol in the wall of the aorta, as well as in the viscera, of herbivorous animals after cholesterol feeding in quantities which they cannot

metabolize, does not produce lesions entirely comparable to those of human atherosclerosis. Perhaps the hypercholesterolemia found occasionally in the late stages of human atherosclerosis may be due to the rupture of atheromatous ulcers with the subsequent discharge of lipids into the blood, and the high lipid content of the blood thus be an effect rather than a cause of atherosclerosis.

An outstanding need at present is a reliable quantitative method for elastin determinations. The disintegration of elastic tissue in atherosclerotic lesions has been observed for a long time morphologically. Does elastin decrease chemically as lipids increase? Pure elastin probably never has been isolated. It is supposedly the protein residue left after all the albumins, globulins and collagen have been removed. In 1911 Selig<sup>8</sup> attempted to compare the elastic content of normal and sclerotic aortas. His methods were crude and he probably did not separate completely elastin from collagen, but he found that with increasing atheroma in the aorta the "protein residue" decreased from over 40 per cent in normal aortas to 10.76 per cent in the most atheromatous ones. In 1913 Ameseder<sup>9</sup> attempted to study the elastin of normal and atheromatous aortas by analyzing for the primary elements composing it, namely, C, H, N, O, and S. He found no significant differences between the normal and the atheromatous vessels in the proportions of these elements. These analyses should be repeated with the more reliable methods now available. If there is no change in the amount of the elements composing elastin as the latter decreases, is it possible that the degenerating, disappearing elastin is converted into lipids? It has been claimed by Walker,<sup>10</sup> Beebe and Buxton,<sup>11</sup> Wells,<sup>12</sup> and others that bacteria can convert proteins into fats, but so far, all attempts to prove that such a conversion takes place in the human body have been unconvincing.

### SUMMARY AND CONCLUSIONS

1. Samples from normal and atheromatous areas of aortas from 11 adult human autopsies were analyzed quantitatively for lipids, and the results compared with microscopic sections from the same areas.

2. The morphological increase in lipids during the progress of atherosclerosis in the aorta was found to be accompanied by a corre-

sponding progressive chemical increase in lipids, including total cholesterol, free cholesterol, cholesterol esters, fatty acids, lecithin and total lipid.

3. The ratio of free to ester cholesterol was found to decrease during the early stages of the process but showed a marked increase in the advanced lesions. This confirms the conclusions of Meeker and Jobling,<sup>2</sup> but is contrary to that of Schönheimer.<sup>4</sup>

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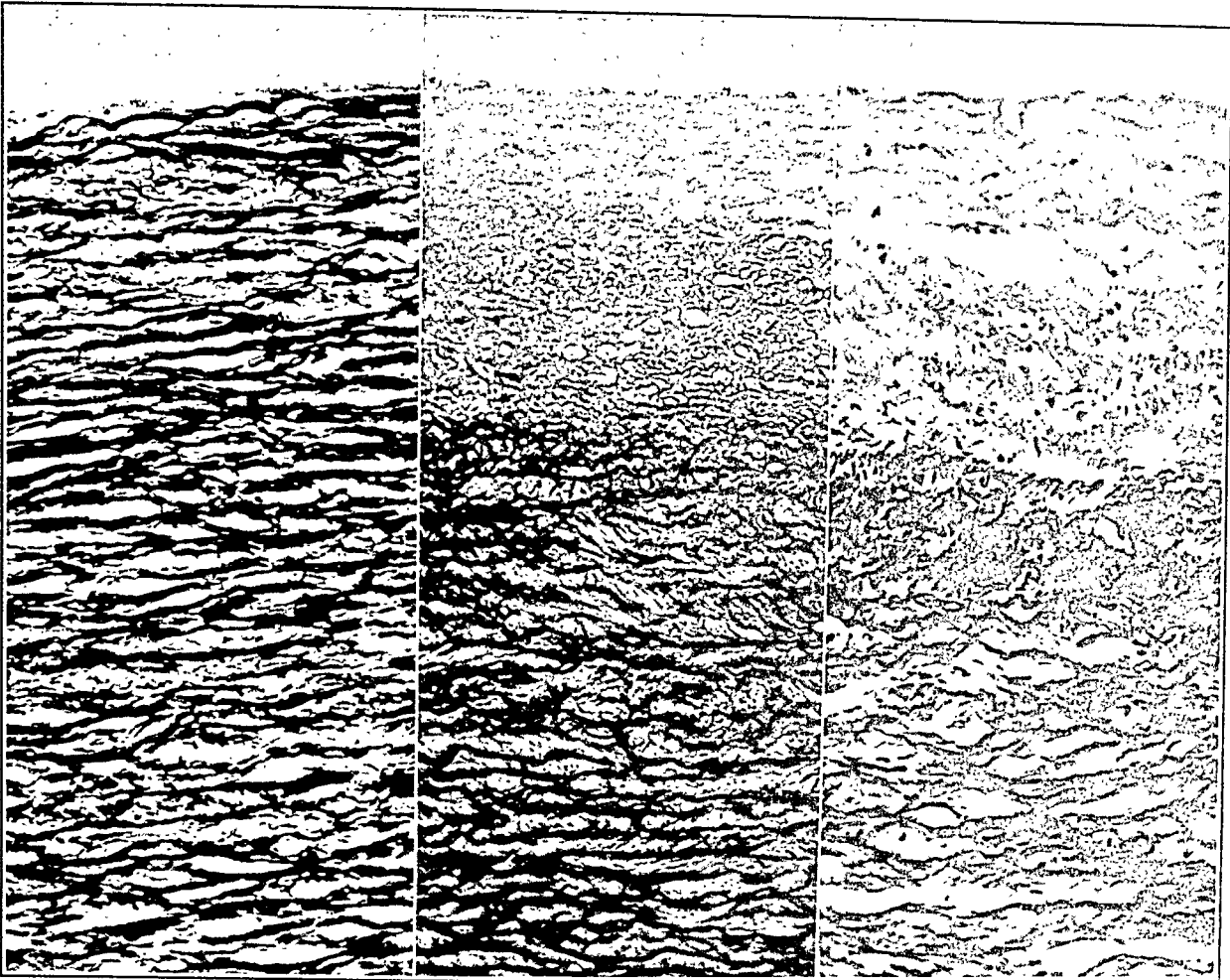
## DESCRIPTION OF PLATE

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### PLATE 16

Types of intimal lesions in aortas analyzed. Verhoeff's elastic tissue stain.

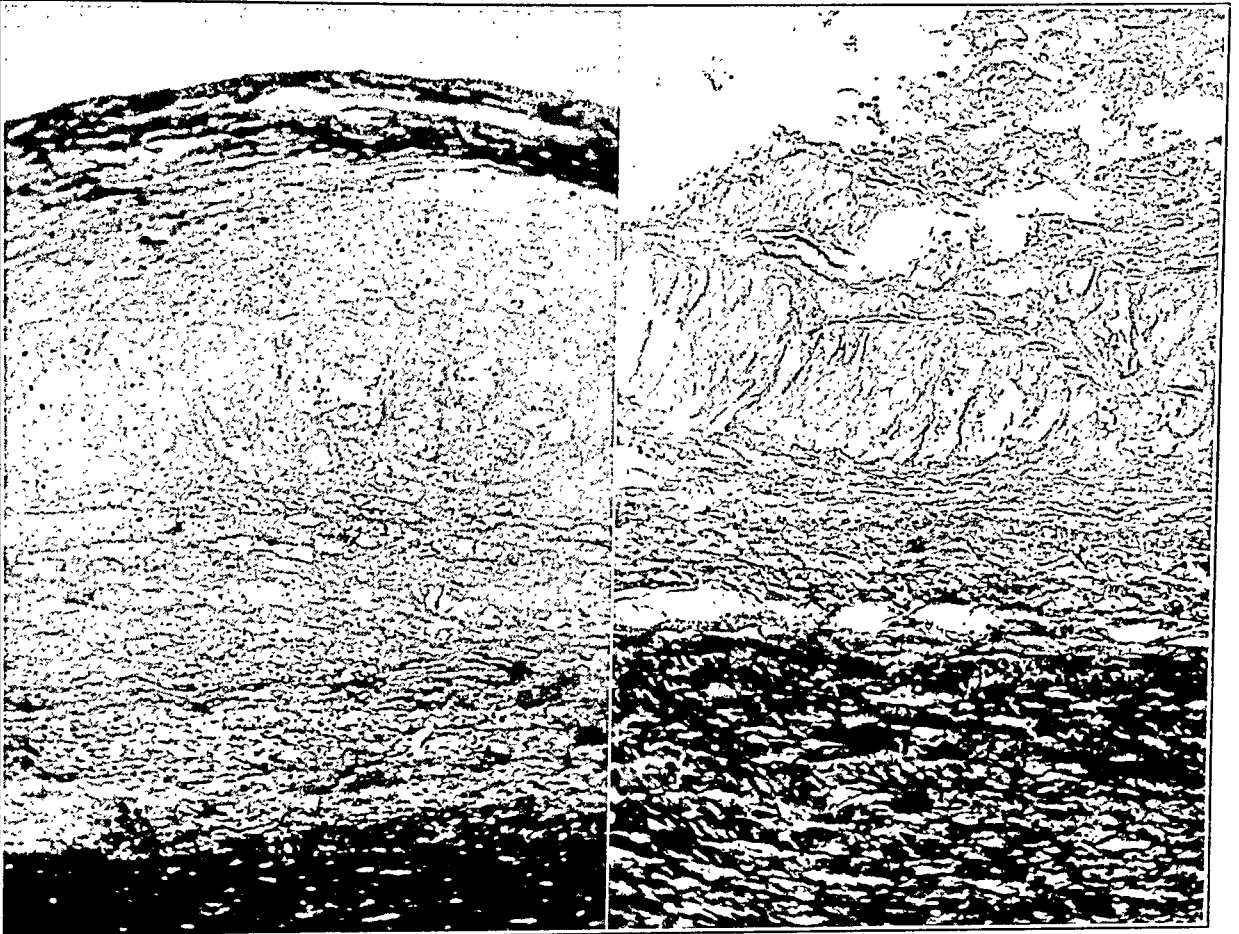
- FIG. 1. Case 4, Area B. No atherosclerosis.  $\times 175$ .
- FIG. 2. Case 5, Area B. Slight atherosclerosis.  $\times 175$ .
- FIG. 3. Case 7, Area A. Moderate atherosclerosis.  $\times 175$ .
- FIG. 4. Case 3, Area C. Marked atherosclerosis.  $\times 75$ .
- FIG. 5. Case 3, Area A. Far advanced atherosclerosis with ulceration.  $\times 100$ .



1

2

3



4

5



# TREPONEMA PALLIDUM IN SYPHILITIC AORTIC VALVULITIS OF A CONGENITALLY BICUSPID VALVE WITH SUBAORTIC STENOSIS \*

## REPORT OF A CASE

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Syphilis of the aortic commissures, associated with mesaortitis, is the usual type of syphilitic heart disease, and lesions do not occur in the body of the valve except by direct invasion from contiguous structures. Only eight adequately described instances of gummatous endocarditis of the aortic valve resulting from invasion of the cusps by a syphilitic process in the root of the aorta, or from a gumma of the interventricular septum, were found in the literature. Involvement, by similar processes, of the pulmonary, mitral and tricuspid valves has been described five, four and three times, respectively, with a histological description of the diseased valve in 10 of these 20 cases (see Table I). A record of the demonstration of the *Treponema pallidum* in a heart valve was not found.

The basis of this communication is a description of a case of gummatous endocarditis of the aortic valve, in which *Treponemata pallida* were demonstrated, and a review of the cases of syphilitic endocarditis found in the literature. In addition to the syphilitic lesions, a congenitally bicuspid aortic valve and subaortic stenosis were present. The concomitance of these two anomalies has been reported in but 3 cases.<sup>1</sup>

## REPORT OF CASE

*Clinical History:* The patient was a 45 year old, white male with clinical evidence of severe aortic stenosis and slight insufficiency, believed to be due to rheumatic disease. Over the upper part of the precordium, maximal in the aortic area, there was a prominent systolic thrill; in the same areas there was a very loud systolic and a blowing early diastolic murmur. The blood pressure was 105/70. A congenital cardiac anomaly was not suspected. There was moderate cardiac decompensation and bronchopneumonia. Substernal pain on exertion and dyspnea appeared 6 weeks before death. The history suggested the pres-

\* Received for publication August 31, 1935.

ence of a valvular cardiac lesion even at the time he contracted syphilis, 15 years before death. The only known antisyphilitic therapy had been six injections of arsphenamine 8 years after the initial lesion. At that time the cardiac signs were similar to those observed during the terminal illness. A history of rheumatic fever was not obtained. The blood and spinal fluid Wassermann reactions were positive. Blood culture was not made and an electrocardiogram was not taken. The temperature was normal.

### POSTMORTEM EXAMINATION

The autopsy, limited to the thorax and abdomen, was performed 31 hours after death.

The heart weighed 525 gm. The epicardium and subepicardial fat were normal. The coronary arteries, which were not tortuous, contained a few small plaques of thickened intima. There were no adhesions in the coronary sulcus. The myocardium showed no areas of scarring. The left ventricle was hypertrophic. All four chambers appeared to be moderately enlarged and contained no ante mortem thrombi. The tricuspid orifice measured 12 cm. and the pulmonic 6 cm. in circumference. The leaflets of both were normal.

The mitral orifice measured 8.5 cm. in circumference. The anterior leaflet was slightly thickened at its free margin, but was not retracted or vascularized. There were no verrucae. The posterior leaflet was normal. The chordae tendineae were slightly thickened and shortened; a few were adherent to each other at their papillary and valvular attachments. The apices of the papillary muscles were slightly atrophic and the endocardial surface was thickened. Scars were not found on section. There was no wrinkling or roughening of the endocardial surface of the posterior wall of the left atrium.

Located below the aortic valve was an elliptical opening, 1.8 by 1 cm., formed by a ring of gray, fibrous tissue, approaching the consistence of cartilage. This ledge, 5 to 6 mm. wide and 2 to 3 mm. thick, was located from 8 to 12 mm. below the base of the aortic cusps at the level of the junction of the ventricular muscle with the fibrous portion of the heart. The base was circumferentially continuous on either side with the mural endocardium of the left ventricle and the ventricular surface of the anterior mitral leaflet. The endocardium between the aortic valve and the subaortic ring was gray, thickened and slightly wrinkled, but contained no endocardial pockets. At the level of this ring there was an abrupt change to a slight

degree of endocardial sclerosis, in the outflow tract. The undefended space was thin, translucent and bulged slightly into the right ventricle.

The aortic valve admitted the tip of the index finger. The orifice measured 8.3 cm. in circumference. An enlarged posterior cusp, located approximately 1 cm. below the level of the margin of the other two cusps, was 2 cm. long and 0.4 cm. thick, and occupied half of the valve orifice as an almost perpendicular, firm shelf of lusterless, reddish gray tissue. The distal four-fifths of the cusp were vascularized and less firm than the proximal portion. There was fusion of the right and left aortic cusps at the anterior commissure; they were of equal size, moderately thickened and retracted, and the edges were rolled outward. Rising above each commissure in the aorta was a bluish white, semicircular, hyaline plaque about 9 mm. in diameter, the surface of which was raised and slightly corrugated. At the posterior commissures these plaques produced moderate separation; the commissural extremities of the posterior cusp were prominent. The hyaline plaque above the anterior commissure did not produce separation but was continuous with a small firm nodule, to which was attached the conjoint segment formed by the fusion of the right and left cusps, along 8 mm. of their margins and 5 mm. of their valve surface. This segment contained four small linear fenestrations. The nodule and margin of the fused cusps formed the raphé of a bicuspid valve. The attachment of the raphé to the sinus wall was slightly above that of the other two commissures. The fusion, firmness and retraction of these two cusps, in addition to the abnormal position and rigidity of the large posterior cusp, produced a slight degree of stenosis in a markedly incompetent valve.

The sinuses of Valsalva were of usual size and contained a few gray and yellow intimal plaques. The ostium of the right coronary artery was narrowed by a semicircular intimal plaque, but the left orifice was normal. The aorta was not dilated. The first portion contained a few areas of puckering and longitudinal wrinkling of the intima extending into the media. Throughout the aorta there was moderate arteriosclerosis.

The remaining significant pathological changes were: severe passive hyperemia of the lungs and abdominal viscera, bilateral hydrothorax and bronchopneumonia.

## MICROSCOPIC EXAMINATION

Blocks of the heart were cut according to the standard method of Gross, Antopol and Sacks.<sup>2</sup> Additional blocks were cut wherever indicated. Sections were stained with hematoxylin and eosin and in duplicate with Weigert's or Verhoeff's elastic and Van Gieson's connective tissue stains. The author is indebted to Dr. C. V. Weller, of Michigan University, for the Warthin-Starry stains, which were done by a slight modification of the method published in 1930 by Farrier and Warthin.\*

The terminology of the valve structure is that employed by Gross and Kugel.<sup>3</sup>

The histological study of the other organs only confirmed the gross diagnoses and revealed no evidence of syphilis.

*Aorta:* The intima of the ascending portion showed moderate, irregular thickening by fibrous tissue, with the formation of a few small atheromata. The media was the seat of perivascular fibrosis and cellular infiltrations; the elastica was disorganized and interrupted. The syphilitic process had caused narrowing of the mouth of the right coronary artery. The descending aorta presented only the changes of arteriosclerosis. Warthin-Starry stains showed an occasional treponema in the supravalvular portion.

*Posterior Commissures:* The same severe type of lesion described in the ascending aorta was present, and in addition a few areas of necrosis and fibrocalcific changes. Numerous treponemata were found in the right commissure, valve ring and annulus near the commissural attachment of the posterior cusp.

*Bicuspid Commissure; Right and Left Aortic Cusps:* There was a raphe composed of dense, avascular fibrous tissue, and areas of elastic fibers cut in various planes and angles, giving a distinct whorled appearance. In addition there were a few areas of laminated elastic and fibrous tissue resembling aortic media. The subendothelial elastica on the surface of the commissure was continuous with the

\* Farrier, R., and Warthin, A. S. A study of the effect of Ph upon the third improved Warthin-Starry method for demonstrating *Spirocheta Pallida* in single sections. *Am. J. Syph.*, 1930, 14, 394-401.

In the method given on page 400 the following changes were used: procedures 5 and 6, 1 per cent solution of silver nitrate (previously 0.5 to 1 per cent solution); procedures 7 and 8, 55° C. (previously 45° C.); procedures 8 and 10, 3 per cent hydroquinone solution (previously 5 per cent); procedure 13 was omitted.

elastica of the aortic intima. These features revealed the congenital origin of this malformed segment. Syphilitic changes were also found here.

The right and left aortic cusps which were irregularly shortened and thickened by fibro-elastic tissue showed no inflammatory changes.

*Posterior Aortic Cusp:* In its proximal half was a large area of coagulation necrosis almost surrounded by fibro-elastic tissue containing epithelioid cells, plasma cells, small and large mononuclear cells and an occasional multinucleated cell. Many of the mononuclear cells contained blood pigment (Fig. 1). Adjacent to this gumma was a smaller area of coagulation necrosis. The proximal fourth and base of the cusp showed a syphilitic process obviously of a more chronic nature, characterized by fibrosis and distortion by large vascularized scars, perivascular cellular accumulations and obliterating endarteritis. There were a few, small scattered areas of calcification. The central portion of the distal half was composed of very edematous, vascularized, hemorrhagic and acellular, fibrous and elastic tissue, and a few inflammatory cells. The subendothelial tissue was less edematous and contained many capillaries, fibroblasts, multinucleated cells, plasma and lymphoid cells. Typical treponemata were demonstrated in large numbers in the distal two-thirds and occasionally in the remainder of the cusp and valve ring (Fig. 2). A Gram stain showed no organisms.

*Subaortic Ridge:* Sections at various places showed an endothelial covered ridge of dense, avascular, almost acellular, hyalinized fibrous tissue containing no inflammatory cells or blood pigment. The intact ventricular elastica beneath the base of this stenotic ledge clearly indicated its congenital origin.

*Section Including Posterior Aortic and Anterior Mitral Leaflets and Subaortic Ridge:* Inflammatory changes extended from the aorta into the base of the posterior aortic cusp and into the valve ring, the annulus fibrosis and tissue immediately behind it, down toward the anterior mitral leaflet, but did not reach the ring of this valve. In this section treponemata were found only in the aortic valve ring. The mitral leaflet presented minor changes consisting of slight thickening with a bulbous tip composed of whorls of dense fibro-elastic tissue in which there was a single small blood vessel. Inflammatory cells were absent.



*Myocardium:* There was severe interstitial fibrosis in the subepicardial tissue adjacent to the larger coronary arteries. Perivascular fibrosis was present throughout the myocardium but the vessels were uninvolved by intimal proliferation or other changes. Aschoff bodies were absent. Warthin-Starry stains of the sections showing the greatest degree of fibrosis were negative.

*Other Sections:* The posterior mitral, the tricuspid and pulmonic leaflets and valve rings were normal. Inflammatory changes were absent in the pulmonary artery. The papillary muscles showed moderate interfascicular and perivascular fibrosis.

### PATHOLOGICAL SUMMARY

*Acquired Lesions:* Syphilitic mesaortitis with narrowing of the right coronary ostium; extension of the process into the right and left posterior commissures and the bicuspid commissures of the aortic valve, and from the aortic root into the posterior aortic cusp, producing severe acute and chronic gummatous endocarditis; interstitial and perivascular fibrosis and hypertrophy of the myocardium. Treponemata were demonstrated in the posterior aortic cusp, in its valve ring, and in the ascending aorta.

*Congenital Anomalies:* Congenitally bicuspid aortic valve and subaortic stenosis.

### DISCUSSION

In syphilis of the aortic valve the inflammatory changes are nearly always restricted to the commissural extremities of the cusps,<sup>4,5,6,7,8</sup> while the free border and body of the leaflets show fibrous, avascular, relatively acellular thickening. The absence of inflammatory changes in the midportion of the cusp serves as a diagnostic distinction from rheumatic valvulitis. When both the commissures and midportion of an aortic cusp are involved by inflammatory changes, a combination of syphilitic and rheumatic disease should be considered.<sup>8</sup> In a rare instance, however, the midportion of an aortic cusp, usually the posterior one, may be involved by extension of an intense syphilitic process of the root of the aorta into the valve ring and base of the cusp, also possibly by a horizontal diffusion from the commissures.<sup>6,7</sup> These are the "ascending" and "descending" types of syphilitic valves described by Benedict.<sup>9</sup> Rarely the syphilitic process in the

root of the aorta may, with or without involving the base of an aortic cusp, extend downward into the anterior mitral leaflet.<sup>10, 11-m, 11-l</sup>

In a gummatous lesion of a cardiac valve the diagnosis of syphilis is evident, even with a negative spirochete stain. Since rheumatic valvular disease is more common than syphilitic, it is necessary, in the absence of giant cells, coagulation necrosis and a positive spirochete stain, to trace the inflammatory changes in the body of a valve leaflet to a syphilitic process in a contiguous structure, usually the aorta or pulmonary artery, in order to demonstrate a presumptive syphilitic etiology.

In the present case the relatively chronic syphilitic process in the proximal third of the posterior aortic cusp was continuous with similar changes in the adjacent commissures, valve ring and root of the aorta, but did not reach the ring of the mitral valve. The most unusual feature is the superimposed acute exacerbation of the syphilitic process in the distal two-thirds of the cusp, and the demonstration of large numbers of treponemata. The latter lesion represents an active syphilitic endocarditis, a condition not previously observed in the 20 cases of valvular syphilis found in the literature. Of the 3 cases with microscopic examination of the aortic valve, Jansen's<sup>11-e</sup> showed histological changes, but obviously of a more chronic nature, most like those in the posterior aortic cusp of the present case. The minor changes observed in the anterior mitral leaflet are evidently not inflammatory and possibly represent changes due to the unusual tension caused by the attached subaortic ridge. The etiology of the interstitial and perivascular myocardial fibrosis is not clear. However, in view of the failure to find Aschoff bodies or any other stigmata of rheumatic fever, the demonstration of treponemata in the aortic cusps, valve ring and aorta gives strong presumptive evidence of a syphilitic etiology. The changes are not unlike some of the less extensive cases of syphilitic interstitial myocardial fibrosis described by Warthin.

The clinical signs of aortic stenosis, caused by subaortic stenosis, far overshadowed those of aortic insufficiency (the principal anatomical lesion of the aortic valve), leading to the clinical diagnosis of rheumatic aortic stenosis and insufficiency in a syphilitic patient who did not give a past history of rheumatic infection. The common stenotic lesion of the aortic valve, even at 45 years of age, is rheumatic aortic stenosis, with or without signs of aortic insufficiency, or

of mitral stenosis. In the absence of clubbing of the fingers, which has been observed in less than half of the cases of subaortic stenosis, there was no reason other than evidence of a severe stenotic lesion at the base of the heart over a number of years, without cardiac symptoms, to suspect subaortic stenosis. The combination of syphilitic aortitis and rheumatic disease of the heart, although recognized to be infrequent, undoubtedly occurs more often than is indicated by the few authentic cases reported in the literature.<sup>8</sup> Combined rheumatic and syphilitic disease of the aortic valve has been diagnosed histologically.<sup>8</sup> Nevertheless, when signs of aortic stenosis and insufficiency are present in a syphilitic patient, the usual diagnosis should be rheumatic disease of the aortic valve. In this patient subaortic stenosis introduces a rare cause of the signs of aortic stenosis in syphilitic aortic insufficiency. Other rare causes of signs of a stenotic lesion at the base of the heart in syphilitic aortic insufficiency are: severe fibrocalcific disease of syphilitic aortic cusps, a condition which cannot be differentiated clinically from rheumatic aortic stenosis; a gummatous aneurysm of the upper portion of the interventricular septum projecting into the outflow tracts; rupture of a syphilitic aortic valve with partial orificial obstruction by the detached cusps; and an aneurysm of a sinus of Valsalva.

Injury to the already damaged aortic valve by the active syphilitic process may have been an important factor in the rapid onset of heart failure. If the presence of a congenital anomaly had been suspected a consideration of subacute bacterial endocarditis would have seemed justified. Angina pectoris associated with disease of the aortic valve is not rare; a frequent cause in syphilis is narrowing of the mouth of the coronary arteries. Such a condition in the ostium of one coronary artery was the probable cause of this patient's anginal syndrome.

#### SUMMARY AND CONCLUSIONS

The case reported herein is the twenty-first authentic instance of true syphilitic endocarditis of heart valves to be found in the literature. It is the ninth case of such involvement of the aortic valve, the process having extended from the root of the aorta into the posterior cusp. An acute exacerbation in the distal portion of this cusp produced subacute syphilitic endocarditis. *Treponemata* were pres-

TABLE I. Cases of Actual Syphilitic (Gummatous or Granulomatous) Invasion of Heart Valves \*

Author	Year	Valve invaded	Origin of syphilitic process	Microscopic examination of invaded valves	Treponema stain
Robinson <sup>11a</sup>	1907	A.V. and T.V.	Gumma of I.S.	....	....
Klages <sup>11b</sup>	1912	A.V.	Aneurysm root of aorta and gumma I.S. Aorta	....	Present in papillary muscle; valve not examined
Spalding and VonGlahn <sup>11c</sup>	1921	A.V.		....	....
Major <sup>11d</sup>	1923	A.V. and P.V.	Gumma of I.S.	....	....
Gallavardin and Josserand <sup>11e</sup>	1927	A.V.	Aorta?	Gummatous endocarditis	....
Jansen <sup>11e</sup> (Case 2)	1927	A.V.	Aneurysm root of aorta	Gummatous endocarditis	....
Norris <sup>11g</sup>	1932	A.V.	Aneurysm of aortic sinus	....	....
Sohval <sup>11c</sup> (Case 1)	1935	A.V.	Gumma of I.S. and root of aorta	Vascularized inflammatory tissue; no giant cells or gummata	....
Author's case	1936	A.V.	Root of aorta	Gummatous endocarditis	Numerous treponemata
Schwalbe <sup>11h</sup>	1890	P.V.	Pulmonary artery	Gummatous endocarditis	....
Stockmann <sup>11i</sup> (Case 1a)	1904	P.V.	Gumma of I.S.	Gummatous endocarditis	....
Holterdorf <sup>11j</sup>	1916	P.V.	Pulmonary artery	....	....
Major <sup>11d</sup> (see above)	1923	P.V. and A.V.	Gumma I.S.	....	....
Kux <sup>11k</sup>	1932	P.V. and T.V.	Pulmonary artery and gumma of I.S.	....	....
Friedman <sup>11e</sup>	1924	M.V.	Aorta?	Large gumma of anterior leaflet	Negative
Staemmler <sup>11m</sup>	1930	M.V.	Aorta	Dense inflammatory tissue with gummata and giant cells	Negative
Sohval <sup>11c</sup> (2 cases)	1935	M.V.	Root of aorta	Dense vascularized inflammatory tissue; no giant cells or gummata	Negative
Robinson <sup>11a</sup> (see above)	1907	T.V. and A.V.	Pulmonary artery	....	....
Bridgman and Schmeisser <sup>11n</sup>	1919	T.V.	Gumma of I.S.	....	....
Kux <sup>11k</sup> (see above)	1932	T.V. and A.V.	Pulmonary artery and gumma of I.S.	T.V. gummatous endocarditis	....

	Aortic	Pulmonary	Mitral	Tricuspid
Cases of syphilitic endocarditis	.....	.....	.....	.....
Cases with microscopic examination of the valve	9	5	4	3
Cases examined for treponemata in valve	4	2	4	1
Demonstration of treponemata in valve	1	0	4	0
Cases in above table with interference of valvular function by juxta- valvular gummata	1	..	0	..
Cases in literature <sup>10</sup> with interference of valvular function by juxta- posed gummata	2	2	0	0
without invasion of valves	.....	.....	.....	.....
Total cases with interference of valvular function	2	6	0	2
	13	13	4	5
A.V. = Aortic Valve P.V. = Pulmonary Valve I.S. = Interventricular Septum T.V. = Tricuspid Valve M.V. = Mitral Valve				

\* In discussing Staemmler's case of mitral gummatous endocarditis <sup>11-m</sup> Geipel mentioned an almost identical case, a detailed report of which was not found. Dr. Maude Abbott, in a personal communication, reported the presence of two cases of aortic gummatous endocarditis in the Medical Museum, McGill University. In both cases there is gross evidence of invasion of an aortic cusp from a gumma of the interventricular septum and one case shows a congenitally bicuspid aortic valve. In the earlier literature descriptions of probable cases of gummatous aortic endocarditis were found. (Corrigan, D. J. On permanent patency of the mouth of the aorta, or inadequacy of the aortic valves. *Edinburgh M. & S. J.* 1832, 37, 225-245. Hope, J. A Treatise on the Diseases of the Heart. Lea and Blanchard, Philadelphia, 1842, Am. Ed. 1.)

ent in the cusp and valve ring. A record of the previous demonstration of treponemata in a cardiac valve was not found.

Congenitally bicuspid aortic valve and subaortic stenosis, two rarely associated cardiac anomalies, were also present. The subaortic stenosis clinically produced signs of aortic stenosis in the anatomical presence of aortic insufficiency, leading to the diagnosis of rheumatic aortic stenosis and insufficiency.

NOTE:—I am indebted to Dr. Robert E. Gross for the photomicrographs and to Drs. Howard T. Karsner and Herbert S. Reichle for helpful suggestions.

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## DESCRIPTION OF PLATE

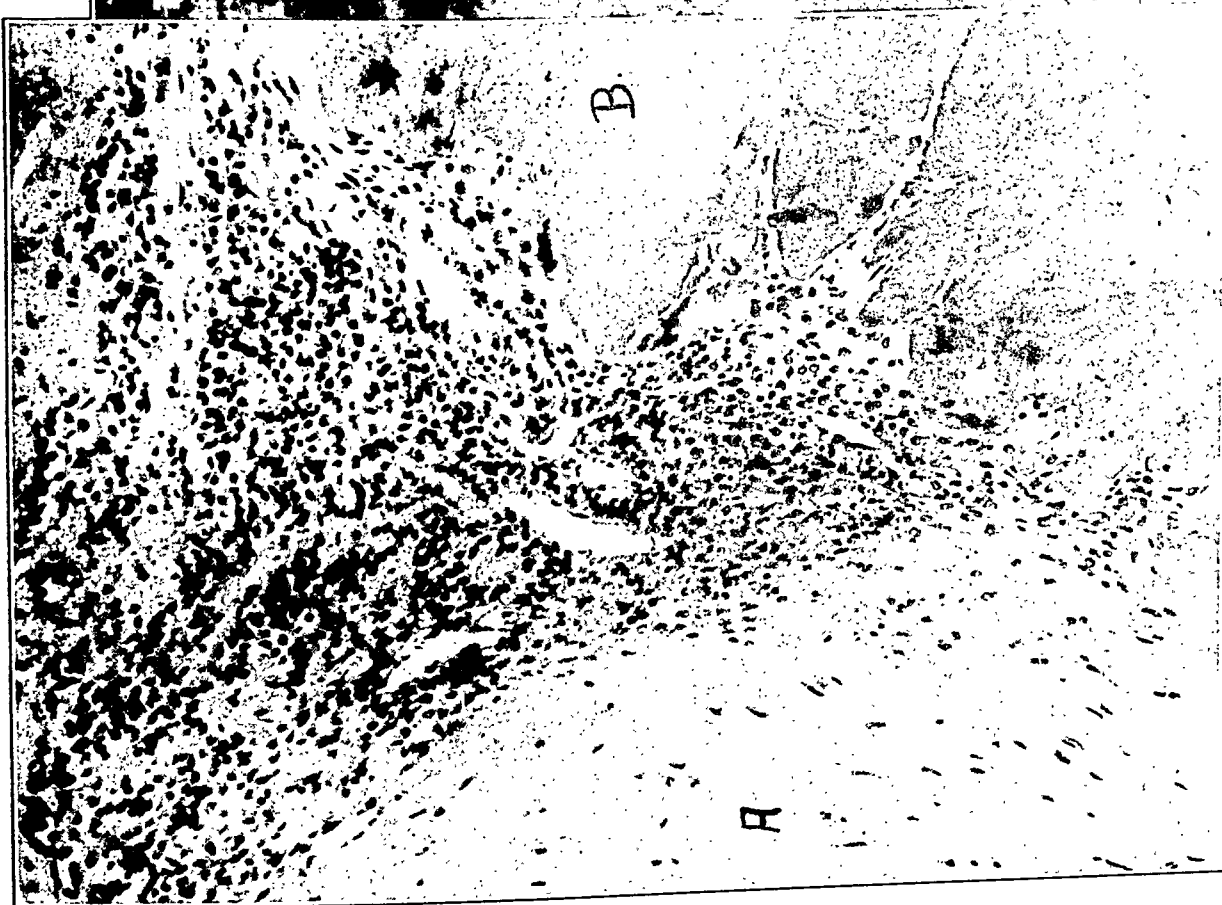
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### PLATE 17

- FIG. 1. Midportion of the posterior aortic cusp showing a part of the gumma. A = Dense fibrous tissue. B = Area of coagulation necrosis. C = Fibroblastic tissue containing plasma cells, epithelioid, lymphoid and large mononuclear cells. Hematoxylin and eosin stain.  $\times 150$ .
- FIG. 2. An area of the distal portion of the posterior aortic cusp showing numerous treponemata, some of which are in the same focal plane. Warthin-Starry stain.  $\times 2000$  (approximately).



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# THE AMERICAN JOURNAL OF PATHOLOGY

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VOLUME XII

MARCH, 1936

NUMBER 2

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## DIRECT BACTERIOLOGICAL EXPERIMENTATION ON THE LIVING MAMMALIAN FETUS \*

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### INTRODUCTION

The concept of parasitism as a general biological problem cannot be complete without a consideration of the reactions of embryonic and fetal animals. The newborn is not a *tabula rasa* upon which the principles of immunology may be inscribed. It is relatively mature in form and function and reflects in large measure the resistance or susceptibility characteristic of its species. We do not know whether these characteristics are manifest in the youngest embryos or become gradually apparent during the intricate growth period from inception to birth. If susceptibility in any degree parallels morphological development, we may expect fetal reactions to differ widely from those of postnatal animals.

Fragmentary information on the occurrence of spontaneous infections of the fetus has accumulated and congenital transmission of many of the infectious diseases is known to be possible. Such transmission is only occasional and usually near term. More data are necessary before we can judge whether the younger fetuses are more resistant to infection or merely less exposed thereto.

Most of the investigations dealing with experimental fetal infections have been concerned with passage of the infectious agent from maternal to fetal circulations through the placenta. In experiments of this type the amount of inoculum that actually reaches the fetus and the time of effective fetal inoculation remain unknown. Many factors affect the result, such as the concentration of the organism in

\* Received for publication September 30, 1935.

the maternal blood stream, the species type of placenta, and the degree of placental damage.

In view of these considerations a more direct approach to the problem of parasitism of the fetus appears desirable. But further than this, there is ample justification on a practical basis for this type of experimentation. Much of the work of the bacteriologist is dependent on the use of experimental animals in the study of both bacteria and viruses. Yet we know that there are definite and characteristic limitations in the use of any particular animal for any given parasite. It is important to determine whether some of these limitations might be modified by extending the host range to include the fetus. Furthermore, the fetus occupies a unique situation, being in an environment of constant temperature and nutrition and well protected from chance contamination and external influence. It constitutes what might be termed a "test tube animal." Such conditions appear ideal for the facilitation of certain types of bacteriological studies.

### TECHNIQUE

Isolated reports of fetal experimentation may be found in the literature published by Charrin,<sup>1</sup> Kreidl and Mandl,<sup>2</sup> Wolff,<sup>3</sup> Bourquin,<sup>4</sup> Bors,<sup>5</sup> Nicholas,<sup>6</sup> Debrunner,<sup>7</sup> Wohlwill and Bock<sup>8</sup> (1933), and others. These studies have been concerned chiefly with pathological changes or with the passage of various substances through the placenta. The work of Bors particularly established the practicability of experimentation on mammalian fetuses.

It is apparent that the purposes of the bacteriologist will not usually require such an elaborate technique as might be demanded for delicate intrauterine surgery in anatomical or similar studies. In the work reported herein we have used the most simple technique consistent with well controlled bacteriological procedure.

Guinea pigs, rabbits and a few rats were used. Most of the work was done on the guinea pig and the remarks herein will refer to that animal unless otherwise indicated. In general, we found the guinea pig the most adaptable to this type of experimentation. They care for their young better, have a longer period of gestation, and tolerate repeated surgical procedures well. The stock animals were kept in large breeding pens and suitable pregnant animals selected from time to time. With a little experience one can judge the approxi-

mate fetal age by palpation. This is possible from about the 20th day of gestation until term at about the 65th day.

Several different methods of inoculation were used, depending upon the fetal age. Until about the 30th day the fetus is so small in relation to decidua, placenta and amniotic fluid that direct inoculation is impracticable. For this stage intraplacental inoculation was employed. The mothers were anesthetized either by drop ether from a separatory funnel, by sodium amytal (Lilly) given hypodermically in a dosage of about 50 mg. per kilo body weight, or by a smaller dose of sodium amytal followed by ether. Following mid-line laparotomy under aseptic precautions, the uterus was delivered, the number and location of fetuses in the two uterine horns determined, and the placentas inoculated by needle puncture through the uterine wall. The abdominal wall was closed in one layer, the skin with heavy interrupted sutures, and collodion was applied to the incision. The placental method of inoculation is necessarily open to the objection that there can be no control of the distribution of inoculum between maternal and fetal blood sinuses in the placenta.

Fetuses from about 30 to 40 days of age were inoculated by needle puncture through the maternal uterine wall and fetal membranes, following surgical exposure of the uterus as described above. At this age the fetuses are readily palpable, the fetal head particularly being easily identified and inoculated. Most of our inoculations were made at this period of gestation and in this way. Needle puncture through the uterine wall is associated with a certain amount of contamination of maternal tissue with inoculum intended for the fetus. In most of our experiments such maternal contamination was not considered important. To avoid this, in certain instances the fetus was exposed by a small incision through the uterine wall and fetal membranes. Following direct needle inoculation the site was cauterized to destroy surface traces of the inoculum.

Older fetuses are more likely to be aborted if the mother is subjected to the full operative procedure. In some cases such fetuses were inoculated through small incisions in the maternal abdominal wall immediately over the fetal part desired. We found it possible also to inoculate older fetuses intracerebrally by needle puncture through the intact maternal abdominal wall. The fetal calvarium becomes calcified long before term and in the later fetal stages can be identified and manipulated against the maternal abdominal wall,

particularly if the mother is well relaxed with anesthesia. Although the danger of perforating some interposed viscus appears great, in no instance was there any evidence that such a thing occurred, nor did any of these mothers abort.

The termination of our experiments was principally by cesarean section. With such a procedure the several fetuses could be identified by their respective positions in the uterus. If the mother is allowed to go to term such identification is not possible and there is the danger that some of the fetuses born dead or alive may be destroyed by the mother. In several instances we have performed serial cesarean sections a week or more apart on the same litter. Occasionally we have delivered mature fetuses by section in order to identify them and then permitted them to be reared by foster mothers. Usually the mothers were sacrificed at section but in many cases the operations were completed surgically, the mothers were re-bred and used again, some of them repeatedly, in similar experimentation.

### EXPERIMENTAL

The following antigens were selected for inoculation in fetal guinea pigs:

- (1) The virus of poliomyelitis, for which the monkey is at present the only susceptible experimental animal.
- (2) The vaccinia virus, which finds a relatively resistant host in the guinea pig.
- (3) The submaxillary gland virus of guinea pigs, a virus natural to that animal and latent in spontaneous infections.
- (4) The tubercle bacillus (H<sub>37</sub>) and Bacille Calmette-Guérin (B C G), representing virulent and relatively non-virulent strains, respectively.
- (5) Diphtheria toxin, for the study of which the guinea pig may be said to be the classical experimental animal.

#### (1) *Poliomyelitis Virus*

A chemically purified and concentrated virus of known infectivity for monkeys was passed through two series of fetal guinea pigs, one at 5 day, the other at 10 day intervals. Eight transfers were made in the former series and four in the latter. The fetuses were 30 to 50 days old. All inoculations were made intracerebrally by needle

puncture through the uterine wall. Fetuses to be used as a source of subinoculation material were removed through cesarean section. From each fetus of the litter one lateral half of the brain was removed aseptically for subinoculation; the other half was preserved for histological examination. The passage material was prepared by pooling the brain tissue from the several fetuses of the litter, grinding with sand in a sterile test tube and suspending in saline solution.

In none of the fetuses were there gross or histological changes that could be interpreted as representing a specific effect of the virus. Material from the sixth subinoculation (5 day series) failed to produce poliomyelitis administered intracerebrally to a monkey. Frequent bacteriological cultures of fetal brain and heart blood in beef heart broth and on blood agar plates were consistently negative.

*Comment:* The above experiments are of course not extensive enough to warrant final conclusions as to the susceptibility of this fetal animal to poliomyelitis virus. Inoculations by other routes, at other ages, through larger series, and in other species would be necessary for complete exploration of the problem. From work reported by Stritar and Hudson <sup>9</sup> on the vaccinia virus, it is evident that the concentration of a virus can vary greatly in the different fetal organs. Perhaps the poliomyelitis virus would find a more favorable medium for growth in some fetal organ other than the brain. Within the limitations of the experiment, however, it is clear that the fetal guinea pig manifested the resistance characteristic of its species to poliomyelitis virus infection. The fact that the fetal tissues remained bacteriologically sterile throughout the course of transfer is noteworthy in view of claims of a bacterial form of the virus.

## (2) *Vaccinia Virus*

A neurotropic strain (Levaditi), maintained in tissue culture, was employed. Its dermal titer in rabbits was 1:10,000. When injected intracutaneously in adult guinea pigs in 1:10 dilution it caused only a faint local reaction and no febrile response. Administered intracerebrally to fetal guinea pigs in 1:100 dilution it was found to disseminate widely and often produced death within 5 to 7 days. The sites of predilection for gross lesions seemed to be skin and lungs, although lesions were occasionally seen in liver, brain and elsewhere. The cutaneous lesions stood out sharply on the hairless fetal skin as

pale, slightly raised nodules from 0.5 to 3 mm. in diameter, some of which progressed to ulceration. The virus could be recovered in high titer from these lesions and its specificity demonstrated by rabbit inoculation.

*Comment:* This is an example of a fetal animal that appears to be much more susceptible than the newborn or adult to a particular virus infection. Although guinea pigs have at times been used extensively in ophthalmic tests for vaccinia virus, and Bland<sup>10</sup> has shown that the virus may be adapted to the guinea pig by serial transfer to the degree that it causes skin lesions, there is a general and well founded belief that the guinea pig is relatively resistant to vaccinia infection.

### (3) *Submaxillary Gland Virus of Guinea Pigs*

Four different preparations of fresh guinea pig submaxillary gland material were injected into fetal and newborn guinea pigs concurrently.\* The results may be summarized by stating that for all four preparations there was a positive correlation between the effects in fetal and newborn animals. One of the preparations was innocuous in both. The other three were effective in dilutions up to 1:1000 in the fetus. Widespread and characteristic lesions and sometimes death resulted. The effects on individual fetuses of a litter were proportionate to the amount of the material injected and the duration of the experiment.

*Comment:* In this instance we are exposing the fetal guinea pig to a virus native to the species yet usually latent in spontaneous infections. Most adult guinea pigs may be considered to possess an "infection immunity," since they manifest no active symptoms of the disease and still carry the virus in an infectious state. Interesting problems in immunology are thus raised. One might expect that immune adults would passively protect their young. However, Markham and Hudson<sup>11</sup> have observed that fetuses can be definitely infected even though borne by spontaneously infected mothers. One may infer, therefore, either that the maternal immunity is not humoral or that antibodies do not pass the placenta in effective quantity.

\* This material was obtained from Dr. F. S. Markham and the inoculations of newborn guinea pigs referred to were made by him (Markham and Hudson<sup>11</sup>).

In these experiments and others the practicability of titration of infectious material in a single litter has been seen. In no case has there been any evidence of transfer of the infectious agent from one fetus to another within the same litter. Control fetuses have never been involved.

#### (4) *Bacillus Tuberculosis* and *Bacille Calmette-Guérin*

The H<sub>37</sub> virulent human strain of *B. tuberculosis*, grown on Long's synthetic medium, was prepared in weighed amounts for inoculation as a suspension in saline. The guinea pig fetuses were injected intracerebrally, for the most part. All such fetuses died within a short time, some *in utero* and others soon after birth. Characteristic changes, both local and metastatic, were evident. Meningitis and necrosis of the fetal brains were often very marked. The liver and spleen usually contained tubercles if the infection had persisted for more than a few days.

The B C G was prepared and injected in like manner. Again, changes characteristic of tuberculosis resulted. Tendency to distribution, however, was much less, and the local lesions were less severe than those produced by comparable dosages of the virulent tubercle bacillus.

*Comment:* Since the guinea pig is a classical animal for tuberculosis work, the susceptibility of the fetal guinea pig is not surprising. Whether the fetus is relatively more susceptible than newborn or adult animals was not determined in these preliminary experiments. They are presented here to illustrate possible advantages of using the fetus in virulence tests. Such experiments have always been open to the objection that the animal may become accidentally infected with extraneous organisms. The fetus, however, is not subject to chance infection, so that the effects of direct inoculation or of serial passage must be given full credence (Neiman and Woolpert<sup>12</sup>).

A point of interest in fetal experiments on tuberculosis was that pathological changes often proceeded *in utero* far beyond the stage that would have been compatible with life in the external environment. The brains of some of these fetuses were almost entirely destroyed, yet the animals lived and developed normally, succumbing promptly, however, when delivered into the outer world as autonomous beings.



(5) *Diphtheria Toxin*

A ripened, standardized toxin, obtained through the courtesy of Parke, Davis & Co., was titrated in 6 newborn guinea pigs and 8 fetuses in three litters. The actual amounts of toxin injected into the fetuses ranged from 0.1 cc. of 1:1280 dilution to 0.1 cc. of 1:20 dilution. All of the fetuses were delivered by section from 2 to 9 days following inoculation. Protocols for the fetal inoculations are set forth in Table I. Table II gives a summary of the results

TABLE I  
*Diphtheria Toxin Titration in Fetal Guinea Pigs*

Guinea pig No.	Approximate days of pregnancy	Day experiment terminated	Fetus No.	Approximate weight of fetus	Inoculum		Results
					cc.	Dilution of toxin	
291	40	49	1	gm. 10	0.10	1:1280	Living; weight 31.2 gm., grossly normal
			2	10	0.10	1:640	Living; weight 31.6 gm., grossly normal
290	35	37	1	5	0.05	1:320	Living; weight 5.5 gm., grossly normal
			2	5	0.05	1:160	Living; weight 6.8 gm., grossly normal
			3	5	0.05	1:80	Dead; weight 6 gm., marked postmortem change
295	38	40	1	10	0.10	1:80	Dead; weight 10 gm., early postmortem change
			2	10	0.10	1:40	Dead; weight 12.5 gm., early postmortem change
			3	10	0.10	1:20	Dead; weight 10.3 gm., extensive postmortem change

computed on a basis of cubic centimeters of toxin per kilogram body-weight of animal and shows that the minimal lethal dose for both newborn and fetus was between 0.05 and 0.10 cc.

*Comment:* These experiments imply that the fetal guinea pig is as reactive as the newborn to the action of this toxin; in fact there is a surprising agreement of titer, considering the small number of animals used. It was shown by Schmidlechner<sup>13</sup> in 1904 that fetal guinea pigs could be killed *in utero* if sufficient toxin were injected into the mother to provide an excess over the amount that would be bound by the maternal tissue. Nevertheless, the impression has

been general that young animals are relatively insusceptible to classical toxins because of a lack of "receptors" or for other theoretical reasons. The fetuses used in our experiments were from one-twentieth to one-fifth the weight of newborn guinea pigs, yet they were acutely sensitive to the small amounts of diphtheria toxin injected. Histological studies were not made. Again, the practicability of titrations in fetal litters is suggested.

TABLE II

*Comparative Reaction of Newborn and Fetal Guinea Pigs to Diphtheria Toxin*

Newborn guinea pigs				Fetal guinea pigs			
0.1	cc. per kilo	—	lethal	0.5	cc. per kilo	—	lethal
0.05	" " "	—	sublethal	0.25	" " "	—	lethal
0.033	" " "	—	sublethal	0.125	" " "	—	lethal
				0.0625	" " "	—	sublethal
				0.0312	" " "	—	sublethal
				0.0156	" " "	—	sublethal
				0.0073	" " "	—	sublethal

## DISCUSSION

Our interest in the fetus as an experimental animal was the outgrowth of efforts to find a more convenient animal than the monkey for poliomyelitis studies. Although this hope failed of realization there appeared sufficient promise in the work to warrant extending its scope to include other infectious agents. The major technical problems encountered were: first, that of inoculating the fetuses without jeopardizing the life of the mother, or producing abortion; and second, that of observing the course of events following inoculation and recovering the virus and fetal tissues at the stage desired. In our experience maternal death occurred rarely and abortion did not commonly take place if healthy mothers at the appropriate gestation stage were used. Graded inoculations and a few trial experiments with the particular virus enabled us to judge fairly accurately the proper time for terminating an experiment. It is hoped that the technique can be modified to permit experimentation on both younger and older fetuses and to include other species. The actual experimental results in this report should be considered as preliminary and suggestive rather than final. More extensive and detailed

experiments are reported elsewhere by Neiman and Woolpert,<sup>12</sup> Stritar and Hudson,<sup>9</sup> and Markham and Hudson.<sup>11</sup>

Any generalizations in theory concerning fetal resistance to infection would be unwarranted at this time. *A priori*, one might favor one or the other of two views: either the fetus in its primitive state should constitute merely good culture medium, devoid of cellular or humoral defense; or, on the other hand, it should be resistant because of its very immaturity. So far as histological reaction is concerned, the impression is general that embryos and younger fetuses are relatively non-reactive. Wohlwill and Bock<sup>8</sup> (1930) could not demonstrate inflammatory reaction in the human fetus under 11.5 cm. in length. From this stage on, a localized histiocytic reaction became gradually evident. Granulocytic infiltration was seen only in the more mature fetuses. In experimental studies the same authors<sup>8</sup> (1933) found that fetal guinea pigs were histologically relatively non-reactive to the bacterial and chemical irritants used. However, lack of histological response does not necessarily imply absence of tissue damage or unsuitability for the support of bacterial growth. We know that embryonic tissues have been found particularly favorable for the propagation of many viruses in tissue culture. In our experience with the fetal guinea pig there were marked tissue changes and often death associated with infection by the tubercle bacillus and the submaxillary gland virus of guinea pigs. The histological changes produced by vaccinia virus were less extensive but death often resulted. Although fetuses were killed by small amounts of diphtheria toxin there was probably little characteristic gross change. The poliomyelitis virus exerted neither vital nor cellular effects. It may be that fetal reactions, like those of adults, will be found to be individualized, with respect to both host and parasite.

#### SUMMARY

A technique for direct bacteriological experimentation on the living fetus of small laboratory mammals is described.

The theoretical and practical advantages of such experimentation are discussed.

The results of preliminary experiments illustrating the applications and limitations of this technique are briefly reported.

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# INTRACEREBRAL INOCULATION OF FETAL GUINEA PIGS WITH BACILLE CALMETTE-GUÉRIN AND THE H<sub>37</sub> STRAIN OF TUBERCLE BACILLUS\*

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## INTRODUCTION

The administration of Bacille Calmette-Guérin (B C G) to infants represents the most popular method in use today for the purpose of protecting man from tuberculosis. It was thought that the production of a subclinical, spontaneously healing infection in man would at least increase resistance to subsequent infection of natural origin. Nocard in 1906 isolated a strain of bovine tubercle bacillus which Calmette and Guérin grew on 5 per cent glycerine-bile-potato medium. They<sup>1</sup> proclaimed this bacterium to be attenuated, after fifteen transfers on this medium, to the degree that it no longer caused progressive tuberculosis in cattle or guinea pigs. Experiments were begun in the protection of animals and by 1922 results were judged so uniformly successful that Weill-Hallé<sup>2</sup> ventured to give the vaccine to infants.

At present B C G is an organism well known to clinicians and investigators interested in tuberculosis. As a vaccine against this disease it is not universally accepted. The controversy centers about the statement made by Calmette that B C G is a "virus fixé," meaning that it is permanently attenuated and not capable of regaining its former pathogenic properties under any environmental conditions. The evidence for and against the ability of B C G to change in virulence by growth on artificial media is not balanced. By far the greater number of workers claim that the known methods of artificial cultivation do not cause B C G to become virulent. In addition, only partial success has attended attempts to produce progressive tuberculosis with this bacterium in experimental animals, as illustrated by the work of Feldman.<sup>3</sup> Transfer of B C G

\* Received for publication September 30, 1935.

from animal to animal has not been accomplished except when a progressive disease has been induced.

Calmette and his supporters have repeated the experiments in the literature purporting to show that B C G is capable under certain conditions of regaining virulence or of producing progressive tuberculosis. The reports of Birkhaug <sup>4</sup> and of Boquet <sup>5</sup> deal with these repetitions and show only negative results. In explanation they affirm that the smooth variant of Petroff, Branch and Steenken <sup>6</sup> and the tuberculosis allegedly caused by B C G are really due to a contamination of the inoculum or to an intercurrent infection of spontaneous origin. In order to eliminate these criticisms, one should use experimental animals known to be tuberculosis-free and maintained in that condition.

The feasibility of using the fetus as an experimental animal has been shown by Woolpert.<sup>7</sup> He has developed a technique whereby fetuses may be inoculated *in utero* without interruption of the gestation period. Such a technique opens many avenues to research and is particularly applicable to the problem of B C G, since the fetus is protected from exposure to intercurrent infection. At most, the remote possibility of transplacental tuberculous infection obtains only when the mother has an overwhelming infection. Another possible advantage of employing this experimental method with B C G is that the fetus may be more susceptible than young or adult animals to relatively avirulent organisms. In this respect also it may constitute a favorable medium for passage experiments in attempts to convert B C G into a more virulent form.

#### EXPERIMENTS WITH THE H<sub>37</sub> STRAIN OF TUBERCLE BACILLUS

It was thought desirable to determine the reaction of the guinea pig fetus to a standard virulent strain of the tubercle bacillus in order to establish a basis for judging the results with B C G. For this purpose H<sub>37</sub> was used. It was originally isolated by Baldwin in 1905 from a case of human tuberculosis and has since been subcultured on artificial media. In this laboratory it has been maintained on Long's synthetic medium.

Accurately determined amounts of tubercle bacilli calculated on the basis of dry weight were injected intracerebrally into fetuses. The age of the culture varied from 25 to 55 days. In inoculating

fetuses by this route it was necessary to pierce the uterine wall and the fetal membranes and calvarium. It is probable that some of the inoculum entered the maternal tissue but gross tuberculosis was rarely observed, being seen in only 1 of 15 mothers whose fetuses were injected with H37. There was no evidence that maternal infection influenced the fetal experimentation.

Thirty-six fetuses in 15 mothers were inoculated with H37. Of these, 13 were alive and 11 were dead when removed by cesarean section; 6 were delivered dead and 2 died several days after delivery; and 4 survived the inoculation of heat-killed bacilli. The dosage varied from 0.01 mg. to 1 mg. The fetuses were removed by cesarean section 5 to 28 days after injection and were examined for signs of life. An autopsy was performed and the tissues were inspected grossly and microscopically. The brains were cultured for tubercle bacilli on Herrold's egg medium and smears of the brains were stained by the Ziehl-Neelsen method for acid-fast bacteria.

*The local reaction* most commonly observed was a meningitis which was infrequently grossly visible. The extent to which the meninges were involved varied directly with the dosage and with the interval between inoculation and examination. Caseous nodules were discernible with the naked eye in the basal meninges in those fetuses delivered dead and in those that died soon after birth (Fig. 1).

In the brains of most fetuses, except those that had received 0.01 mg. and were examined in the early stages, an internal hydrocephalus was present. Grossly it was impossible to determine where the obstruction to the flow of cerebrospinal fluid was taking place, but many microscopic sections disclosed tubercles at the base of the brain, extending into and occluding the foramina of Luschka (Fig. 1).

*The dissemination* of the tubercle bacilli to other organs was followed histologically in sections stained by the Cooper modification of the Ziehl-Neelsen technique.<sup>8</sup> About 3 weeks after inoculation with all dosages, gross tubercle formation was noted in the liver, spleen and lungs. Microscopically it was found that the liver was the first organ to be affected. Bacilli appeared here as early as 5 days after intracerebral inoculation even with the smallest dose. The next organ to be involved was the spleen, in which the appearance of bacilli was variable. Sometimes they appeared early in the course of the infection and at other times late. Some fetuses inoculated with as much as 1 mg. of H37 did not show the bacilli in the spleen at



H37. The B C G was kept in a separate incubator from that in which the cultures of H37 were grown. Likewise, animals whose fetuses received the former were caged in a different room from those whose fetuses were inoculated with H37.

*Inoculations of 1.4 to 3 mg. Amounts of B C G:* These were made intracerebrally into fetal guinea pigs of varying ages. They were examined for signs of tuberculosis after certain time intervals. The large doses were used in initial experiments because of the purported avirulence of B C G for adult guinea pigs. Seven fetuses in 3 mothers were inoculated through the maternal abdominal wall with 3 mg. of the bacilli from 5 to 7 days before delivery. All the fetuses died of generalized tuberculosis from 1 to 45 days after delivery. Ten fetuses in 3 mothers were inoculated, after aseptic laparotomy to expose the uterus, with 2 mg. of this bacterium. Four died of generalized tuberculosis immediately after birth. The remaining 6, when examined *in utero* 2 weeks after inoculation, were found to be tuberculous although alive. Three fetuses in 1 mother were injected with 1.5 mg. They were removed by cesarean section 8, 14 and 20 days after inoculation. The tuberculosis was found to be progressively greater in each fetus, the last being dead when removed. Four fetuses in 1 mother were inoculated with 1.4 mg. and died of generalized tuberculosis immediately after delivery, 31 days after inoculation. The diagnosis of tuberculosis in these fetuses and newborn was based on gross and microscopic evidence.

*Experiments with Smaller Amounts of B C G:* The results of the above experiments were attributed to large doses. Accordingly, a series of 35 fetuses in 16 mothers were inoculated by the same technique described for H37 with doses ranging from 0.01 mg. to 1 mg. Only 5 fetuses were inoculated with 0.01 mg. because this dose was early found to produce very little pathology. Three fetuses receiving this amount were delivered naturally. One was dead at birth but showed no tuberculous changes; 1 was killed and autopsied at 6 months of age and found to be essentially normal; the other is still alive (15 months after inoculation). Two fetuses were examined *in utero* 14 days after inoculation. Both were alive; 1 was apparently normal in every respect, and in the other there were minor histological changes.

Twenty-seven fetuses were inoculated with 0.1 and 1 mg. amounts of B C G. Of these, 2 were killed 2 days after delivery (35 days

after inoculation), 2 died immediately after birth, and 1 died 1 day and another 4 days after delivery. All of them showed generalized tuberculosis at autopsy, manifested by caseous nodules in the brain and liver, and a gray and nodular spleen. A single animal that received 0.1 mg. of the inoculum is still alive 1 year after inoculation.

The 20 remaining fetuses were removed alive by cesarean section from 7 to 28 days after inoculation. The only gross change locally was a distention of the lateral ventricles, which was explained in most instances by a microscopic tubercle occluding the foramen of Luschka. Also, microscopically a meningitis was demonstrable in most of the fetuses. Only with the larger dosages and after the longer intervals were tubercles found in the brain tissue proper.

*The distribution* of the inoculum to other organs was followed histologically and it was found that the liver was the most frequent site of metastasis. The lungs and spleen were often sites of deposition of the bacilli but the placenta was only infrequently involved. However, dissemination was not as rapid as seen with H37. Those fetuses inoculated with 0.1 mg. did not show tuberculous involvement of organs, aside from the brain, except after the longer intervals, but those receiving 1 mg. manifested a rapid spread. In Table I these findings are summarized and compared to the observations made on H37 in this respect.

*Controls:* Nine fetuses were inoculated with heat-killed bacilli. All were delivered alive and apparently normal, but 3 died soon after birth. Autopsy showed an internal hydrocephalus and a localized meningitis in which no tubercle bacilli were demonstrable. All the other organs were essentially normal. No tuberculous pathology was found in the other 6 when they were killed and autopsied 6 months after delivery.

*The tubercles* found in the fetuses receiving B C G had the same cellular structure and showed the phenomenon of satellite tubercles as described for those produced by H37. The acid-fast bacilli had the same microscopic appearance as H37 and were extracellular as well as intracellular (Figs. 4, 5 and 6). They were in the large mononuclear cells in such great numbers that it was impossible to interpret their presence except on the basis of their multiplying in the cytoplasm of these cells.

*Recovery of B C G* on artificial media met with only partial success. It was grown out on Herrold's egg medium in 2 cases from

fetuses inoculated with 3 mg. and from 2 fetuses injected with 1.5 mg. In twenty other attempts the bacilli were not recovered. The organisms did not seem to have changed in colony characteristics, morphology or staining reaction from this single passage. However, it did take a longer time for an abundant growth to appear, that is, 6 weeks as compared with 15 to 20 days for the original culture.

*Fetus to fetus transfer* of B C G was attempted on three occasions. Two of these are to be disregarded because the fetuses receiving the first subinoculation were aborted soon after injection. On the third attempt, however, three subinoculations were performed. The fetuses of 2 pregnant guinea pigs were inoculated with 0.1 mg. of bacteria. Three weeks later all the fetuses were removed under strictly aseptic conditions. The brain of one was ground up in a sterile mortar with 5 cc. of saline, lightly centrifuged and a smear made of the supernatant fluid. There were 10 acid-fast bacilli per oil immersion field. The following disposition of the material was made.

(1) 0.25 cc. was inoculated into the groin of each of 2 adult guinea pigs.

(2) Three tubes of Long's and one flask of Sauton's media were seeded.

(3) 0.1 cc. was inoculated into each of the fetuses of a pregnant guinea pig.

The adult guinea pigs killed and autopsied 6 months later showed no tuberculous changes although there had been a slight enlargement of the inguinal lymph nodes at first. No growth appeared on the media. The guinea pig whose fetuses had received the subinoculation was found 9 days later to be rupturing through the site of incision. An aseptic laparotomy was immediately performed and the fetuses removed. The brain of one was treated precisely as that of the initial fetus and the material used for the second serial transfer. However, no acid-fast bacteria were found in the supernatant fluid after centrifugation.

Adult guinea pigs inoculated with this material were negative for tuberculosis when autopsied 6 months after inoculation. No bacteria were recovered in culture. The fetuses were examined 3 weeks after inoculation but no evidences of tuberculosis were found. No tubercle bacilli could be demonstrated in histological sections of the fetuses from both the first and second transfers.

A third serial inoculation to adult animals, media and fetuses was made, again with negative results.

*Summary:* B C G was inoculated intracerebrally into fetal guinea pigs in doses ranging from 0.01 to 3 mg. The animals were examined for gross and microscopic pathology after removal by cesarean section or after birth. Most of the guinea pigs exhibited different stages of tuberculosis, depending on the dose and the time interval. A few of those receiving the smaller dosages manifested no evidence of tuberculosis. Many of the animals that were permitted to be born naturally died of generalized tuberculosis or showed extensive tuberculous changes when sacrificed.

Recovery of the organisms on artificial media was only partially accomplished and the few attempts at fetus-to-fetus transfer were unsuccessful.

TABLE I

*Dissemination of Tubercle Bacilli in the Guinea Pig Fetus After Intracerebral Inoculation*

Dosage	Fetuses inoculated with H37				Fetuses inoculated with B C G			
	Total	Number essentially normal	Number tuberculous		Total	Number essentially normal	Number tuberculous	
			Only at site of inoculation	In other organs also			Only at site of inoculation	In other organs also
mg.								
0.01 .....	13	0	0	13	5	4	1	0
0.1 .....	12	0	0	12	13	1	7	5
1.0 .....	7	0	0	7	14	1	2	11
1.4 .....					4	0	0	4
1.5 .....					3	0	0	3
2.0 .....					10	0	0	10
3.0 .....					7	0	0	7
Totals ...	32	0	0	32	56	6	10	40

Percentage showing dissemination of tubercle bacilli

100 per cent

71.4 per cent

## DISCUSSION

Table I summarizes the incidence of dissemination from the site of inoculation of B C G, as compared with H<sub>37</sub>. The latter bacillus was able to establish foci of infection in distant organs even with the smallest dosage. The B C G was able to spread too, but only when inoculated in the larger amounts, 0.01 mg. having hardly the ability to establish a local focus. However, too much weight is not to be placed on this fact since it was in the fetuses examined after the shorter periods that only local changes were found. Furthermore, it is the fetuses inoculated with the smaller dosages and allowed to go for the longer periods that fall into the last column of Table I. The gross and histological pathology produced by these agents in fetal guinea pigs was similar in all essential respects. In addition, the two types of bacilli in stained sections appeared morphologically identical.

Care must be exercised in judging the relative virulence of bacteria on the basis of histological evidence. The fact that B C G was found to spread from the site of inoculation is not sufficient to stamp it invasive. Even avirulent or dead bacteria inoculated by various routes may be scattered through the body by way of the blood stream. However, a certain degree of virulence and growth is implied when tubercle bacilli are associated with lesions showing various stages of development, are intracellular in large numbers, and assume a definite relation to one another extracellularly as well as intracellularly. Judged by these criteria, B C G seems to be definitely pathogenic for guinea pig fetuses. Furthermore, the similarity in the histological pictures produced by B C G and by H<sub>37</sub> supports the same idea. Why B C G is relatively virulent for fetal guinea pigs and what the general significance of this fact is cannot be discussed here.

Our failure to pass B C G from fetus to fetus in the few attempts made appears to be inconsistent with the extensive pathological changes produced, yet it falls into line with work on adult animals reported by Feldman<sup>3</sup> and others.<sup>10</sup> A possible explanation lies in the factor of dosage since in the method employed there was no attempt to concentrate the inoculum.

The difficulty in recovering B C G on artificial media, also noted by Feldman,<sup>11</sup> perhaps may be explained by an insufficient

amount of inoculum. Control inoculations of the same media were made, using bacillary suspensions from cultures and containing the same number of organisms as in the fetal material subinoculated, that is, 10 acid-fast bacilli per oil immersion field. No growth took place, although tubes of the various media employed seeded directly with the dry organism showed abundant growth in 3 weeks.

It appears essential that one should be able to transmit B C G from fetus to fetus and to recover it on artificial media in order to establish its activity. More extensive experiments along this line are in progress.

### SUMMARY

Guinea pig fetuses were inoculated with graded doses of B C G and the effects compared with those obtained in a similar series inoculated with virulent tubercle bacilli (H37). Both types of tubercle bacilli were found to spread from the site of inoculation but not with equal rapidity; with the same dose, it took a longer time for B C G to cause as much pathology, in the animal as a whole, as H37. Pathologically the response of the fetus to both organisms was the same. Histological evidence has been brought forward to show that B C G is capable of multiplying in fetal tissues and of initiating a disease process different only in degree from that produced by H37. So far, attempts at fetus-to-fetus transfer of B C G have not been successful. Recovery of this bacillus on artificial media from inoculated fetuses was accomplished four times in twenty-four attempts.

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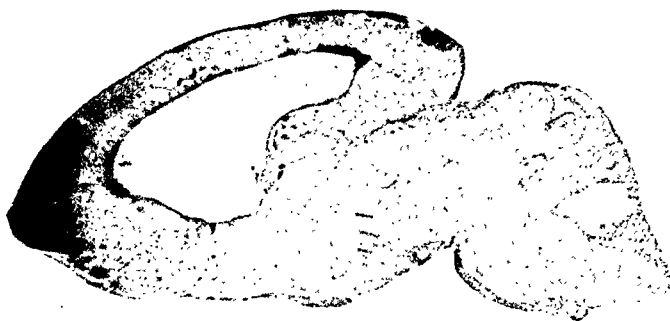
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## DESCRIPTION OF PLATE

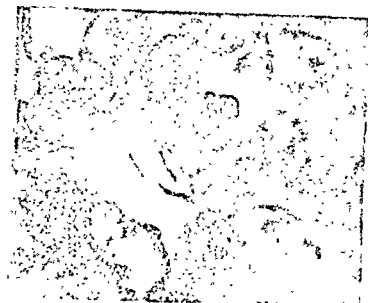
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### PLATE 18

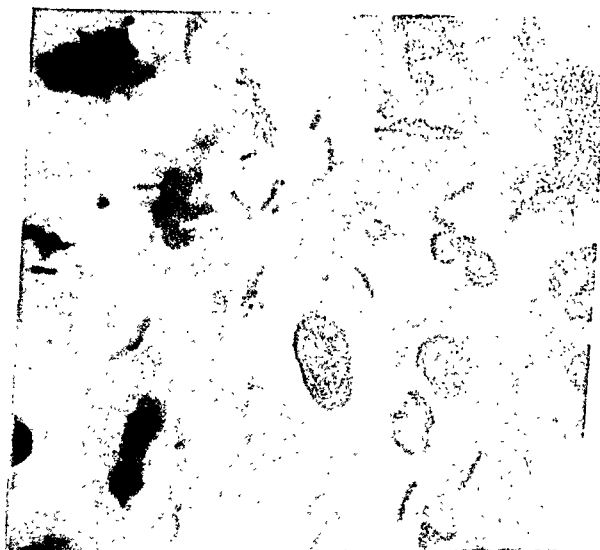
- FIG. 1. Longitudinal section of brain of fetus 21 days after inoculation with 0.01 mg. of H37, Ziehl-Neelsen stain. Note tubercle occluding the foramen of Luschka and the resulting enlarged cerebral ventricle. Typical meningitis and inflammatory areas in walls of lateral ventricle.  $\times 2$ .
- FIG. 2. High power photomicrograph of meningitis in base of brain of Fig. 1, Ziehl-Neelsen stain. Typical granular tubercle bacilli.  $\times 1500$ .
- FIG. 3. Section of placenta from fetus 21 days after intracerebral inoculation with 0.1 mg. of H37, Ziehl-Neelsen stain. Early tubercle associated with older tubercle (not shown). Polymorphonuclear cells are present with the large mononuclear cells beginning to appear.  $\times 1000$ .
- FIG. 4. Section of brain of fetus 21 days after inoculation with 0.1 mg. of B C G, Ziehl-Neelsen stain. Large masses of acid-fast bacilli in the cytoplasm of large mononuclear reacting cells.  $\times 1500$ .
- FIG. 5. Section of brain from same fetus as in Fig. 4, showing acid-fast bacilli lying parallel extracellularly.  $\times 1500$ .
- FIG. 6. Section of brain of fetus 7 days after inoculation with 0.1 mg. of B C G, Ziehl-Neelsen stain. Typical large mononuclear cell lying free in lateral ventricle, the cytoplasm filled with acid-fast bacilli.  $\times 1500$ .



1



2



3



4



5



6





# SUSCEPTIBILITY OF THE GUINEA PIG FETUS TO VACCINIA \*

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## INTRODUCTION

Vaccinia is one of a group of virus diseases that under natural conditions present lesions of similar appearance in a variety of animals. The experimental host-range of vaccinia itself is also wide, including man, monkeys, cattle, fowl and rodents. Animal susceptibility is not necessarily a property common to the members of an allied group, however, for the receptivity on the part of a single member may be of only a relatively mild degree. The response of the guinea pig to vaccinia is an example; while the rabbit is highly susceptible, the former is generally regarded as comparatively non-reactive.

The cultivation of the vaccine virus in tissue cultures has introduced a semi-artificial condition in which cells even of embryonal nature serve as a medium. The demonstration of this principle by Rivers<sup>1</sup> and his associates has been extended more recently by Goodpasture, Woodruff and Buddingh<sup>2</sup> to the successful cultivation of the virus in membranes of the chick embryo. They have found a greater susceptibility of the embryonic tissues than of the hatched chick to vaccinia.

Various agents capable of inducing disease have been reported as more invasive in avian embryonic tissues than in hatched or adult birds. As long ago as 1912, Murphy and Rous<sup>3</sup> reported that their chicken sarcoma grew more favorably in the embryo (chicken, pigeon, duck) than in the adult. The same has been found true by Rivers and Schwentker<sup>4</sup> in the case of a parrot disease virus. Mackenzie,<sup>5</sup> studying Rift Valley fever, Burnet,<sup>6</sup> working with canary pox, and Syverton, Cox and Olitsky<sup>7</sup> in equine encephalomyelitis experiments, likewise noted the growth of their respective viruses in chick embryo tissue cultures while young or adult fowl were not responsive.

\* Received for publication September 30, 1935.

Woolpert,<sup>8</sup> in seeking to demonstrate any peculiar susceptibility of the fetus in a species whose adults are relatively resistant, found in preliminary experiments that the guinea pig fetus reacted to vaccinia virus. We have investigated the problem and this paper reports our studies on the susceptibility of this type of experimental animal to the virus. In short, it was found that lesions appeared, death sometimes ensued, and the virus increased in the fetal animal, while the same virus had little or no effect on the guinea pig after birth.

## EXPERIMENTAL

### *Materials and Methods*

The neurovaccine strain of Levaditi was used throughout these experiments. It was carried free of bacteria by testicular passage in rabbits, and its virulence, as measured by the ability to produce cutaneous lesions in the same animal, was constantly maintained. The operative technique was in general as described by Woolpert.<sup>8</sup> Most fetuses were inoculated intracerebrally, only a few intraplacentally.

About a third of the fetuses were injected with rabbit testicular material, the remainder receiving suspensions of guinea pig fetal tissues proved to contain virus. The potency of testicular and fetal virus was regularly determined by rabbit skin titration. The titer varied from 1:100 to 1:100,000, three-fourths the preparations having a titer of 1:10,000 and 1:100,000. The fetal virus usually measured 1:10,000 and the testicular material was somewhat more potent. The doses used were 0.1 cc. for each fetus inoculated, and when a preparation was injected in a number of fetuses it was commonly employed undiluted and diluted 1:10 and 1:100.

Fetuses in various stages of gestation were employed. The range was from 27 to 53 days of intrauterine life, the average was 39 and the median 37 days. The experimental period varied widely, being from 2 to 35 days; the majority of experiments were terminated between 5 and 7 days after inoculation. In addition to delivery of the fetuses by cesarean section, experiments were ended by such factors as threatened or actual abortion, birth, or fetal resorption. In a few instances the young that were born alive furnished other material

for examination. Excluding the fetuses that were not available for study because of early abortion, intrauterine maceration, resorption or calcification, and maternal destruction of aborted fetuses, data were obtainable on 115 fetuses inoculated with potent vaccine virus.

Controls on the virus employed in inoculation consisted of repeated demonstration of cutaneous vaccinia lesions in the rabbit and their prevention by mixture with antivaccinia rabbit serum. Furthermore, the results in the fetus were controlled by the recovery of the virus from fetal organs by rabbit skin inoculations and again its neutralization by specific immune serum. Occasionally a fetus of a litter was uninoculated and served as a control on the infectiousness of the material used in its litter mates. All operative procedures were carried out with strict aseptic technique and infrequent bacteriological examinations of the inoculum and the tissues of infected fetuses were negative.

Throughout the fetal experimentation the virus was repeatedly tested for its action on adult guinea pigs. The rabbit testicular virus was tested by the cutaneous and intracerebral routes, while the infected fetal tissues, controlled by rabbit skin reactions, were examined by the cutaneous, intracerebral, intravenous and intratesticular routes. The only observable lesion elicited in the guinea pig was a mild hyperemia with papule formation at the site of intracutaneous inoculation. This appeared only occasionally and with the highest concentration of inoculum. In no instance was a febrile reaction obtained. These results indicate the relative non-reactivity of the adult guinea pig to the test virus employed in the fetal experimentation and furnish a basis for comparison with the results to be described.

## RESULTS

The action of the virus on the fetus could best be judged by the gross and microscopic pathology. Death as a criterion of virus effect was not dependable, since fetal mortality may result from natural causes and we are not aware of its incidence under ordinary conditions. In our experimental series dealing with 80 mothers there were 10 fetuses that were found in various stages of resorption, dehydration and calcification. This is not a complete picture, however, as no doubt many killed by virus action were aborted and lost.

### *Gross Pathology*

The lesions seen in the infected fetuses appeared in the skin and various viscera. Because of the small size of the organs, the presence of lesions was judged mainly by the external changes in each part. For the same reason, pathology in the intestines was not definable. Changes were recorded in various parts of 88 of 115 fetuses inoculated with virus proved potent by rabbit skin test and observed under conditions suitable for detection of lesions. The 27 fetuses not showing any gross pathology included those injected with high dilutions of virus material. Of the 88 animals, lesions were seen in the skin in 33, on the surface of the brain in 11, in the lungs in 54, in the liver in 25, in the kidneys in 55 and in the spleen in 9 instances. The placenta and heart showed gross changes in each of 2 fetuses. It is clear that the distribution of lesions was irregular and that they occurred most commonly in the lungs and kidneys.

The lesions in most parts were circumscribed and usually with a regular margin. They varied in size from being barely discernible to the naked eye to 2.5 mm. in diameter. The largest number of lesions were flat, although a few were elevated in the form of vesicles and some were depressed or pitted. The kidneys of 3 fetuses were marked by small ulcerating foci. Most of the lesions were gray or gray-white and with a sharp outline; these occurred in all the tissues but were most conspicuous in the skin. Minute hemorrhagic areas appeared on the lung surfaces in 11 fetuses, and occasionally irregular areas of hemorrhage 2 mm. in diameter surrounded by diffusely pale grayish tissue were found involving the kidney. Rarely, gray lesions elsewhere were tinged with red or surrounded by narrow zones of hemorrhage. In general, no differences in the gross changes in individual sites were attributable to the source of virus, although lesions of the brain were more common in the fetuses inoculated with rabbit material, and splenic alterations were seen only in the animals that received fetus virus. Representative lesions are pictured in Figures 1 to 7.

### *Microscopic Pathology*

The microscopic features characterizing the acute effect of vaccine virus on the fetal tissues are necrosis, edema and hemorrhage. There is a noteworthy lack of cellular infiltration, although in older lesions

a mesenchymal reaction is apparent. The detailed study of the histopathology will be made the subject of another communication.

### *Vaccinia in the Fetus*

Thirty-three of the inoculated fetuses were examined in detail for the effect of vaccinia. Suspensions of their tissues were reinoculated into rabbit skin for the identification and titration of virus and to determine its distribution in the fetus. Twenty-six had been injected with suspensions of infected fetal organs (15 with renal, 7 with brain, 2 with lung and 2 with placental suspensions) and 7 received rabbit testicular virus. The results cannot be correlated with the type of material injected and the data will be considered together. Further, the titer of the virus in the organs examined was not referable to the strength of the original inoculum. The period varied widely, as previously indicated; the tissues of 22 of these fetuses were examined from 4 to 7 days after inoculation. The duration of the experiment under these conditions did not seem to have a direct bearing on the demonstration of virus and its distribution and titer.

Vaccine virus was recovered from fetuses by rabbit inoculation and the rabbits showing positive skin reactions to this virus were proved immune to subsequent infections of virus of rabbit origin. Virus was demonstrated in all but 3 of the 33 fetuses, and in 2 of these, lesions in the lungs were apparent. Virus was recovered from 18 of 24 brains, 20 of 31 lungs, 13 of 26 livers, 6 of 17 spleens, 23 of 26 kidneys, 2 of 3 skin lesions, and 11 of 17 placental tissues tested. The blood of 10 fetuses was examined and only 2 yielded virus and they in low dilution. In all, suspensions of 146 organs (excluding blood) were tested quantitatively in tenfold dilutions. Ninety-four gave positive results in the following titers: 13 to a titer of 1:1; 10 to 1:10; 36 to 1:100; 15 to 1:1000; 18 to 1:10,000; and 2 to 1:100,000. Not only did the kidney furnish the highest incidence of virus recovery, but it also more often gave the highest titer among the organs, usually being 1:10,000. It was used most frequently as a source of passage virus.

When the incidence of virus recovery was compared with the presence of lesions in various parts, it was observed that of the 146 tissues examined for virus, lesions and virus both were found in 57 tissues, neither was in 37, the virus and no lesions were in 39, and

lesions but no virus were demonstrable in 13. In this series of 33 animals, gross changes were thus noted in 70 tissues. In 3 fetuses no pathology was seen, but in 2 of them the virus was isolated from some part. The virus was found to persist to the limit of the experimental periods and was recovered as late as 32 days after fetal inoculation. Likewise, lesions were observed after the maximum interval of experimentation.

### *Fetal Source of Virus*

We have already indicated that the virus of fetal origin behaved in fetuses as that of rabbit passage in all the respects noted in the experiments. That the virus increased in the fetus was demonstrated by the effective transfer of virus through series of fetuses. In one group the fetuses of 9 mothers were serially inoculated, beginning with 1:100,000 titer of testicular neurovaccine and ending with 1:10,000 titer of virus in the fetal kidney, with lesions present. Besides establishing the multiplication of vaccinia virus with the development of lesions in the fetus, it was found that the highest concentration and the most frequent effect of the virus was in the kidneys. With all this, however, there seemed to be no selective adaptation of the virus to the fetus, since the virus was constantly transmissible to the rabbit with typical manifestations, its characteristics in the guinea pig fetus did not change during the year of experimentation and it was not modified in its relative non-infectivity for the adult guinea pig.

### *Vaccinia in the Newborn*

All gradations of effects were noted among the 35 animals that were delivered at term after inoculation *in utero*. Six died soon after birth and some displayed lesions with virus demonstrated in low titer in rabbit skin. Nine others lived from a few days to 3 months; of these, 2 had vaccinial lesions in the lung and kidney, 3 showed softening of the cord and brain after symptoms of paralysis and weakness, and the others died showing pathology not referable to experimentation. The remaining 20 animals survived. Although the skin in all animals born was examined thoroughly, often from the undersurface as well, no lesions were seen in the group that was born alive.

The susceptibility of the newborn was tested, with the same non-reactivity resulting as was obtained in the inoculation of adult guinea pigs. Eleven animals from 4 to 10 days old were injected intracerebrally with potent virus and none showed fever or symptoms.

## DISCUSSION

Much experimental work has been done on the parasitization of mammalian hosts with viruses. The range of animals in which disease is induced has been greatly extended by the introduction of special techniques, as well as by the use of animals not naturally exposed to specific infection. Little has been done, however, on the susceptibility of the mammalian fetus. The whole question of fetal reactivity has been only slightly explored, due perhaps to hesitancy in operative procedures. Although there are definite restrictions on account of the essential circumstances, the field is worth exploring on the bases of parasitism and fetal physiology.

We are here reporting the successful propagation of the virus of vaccinia in the guinea pig fetus, with the development of typical lesions. This prenatal susceptibility, as compared with the relative resistance of the adult, is consistent with the work already cited of the successful growth of viruses in cultures of embryonic tissues. Such cultures apparently furnish a circumstance sufficiently favorable for intracellular growth. That this is possible in tissue cultures of the natural host is logical, but evidently some other factors enter that allow virus growth in the fetus whose adult is relatively insusceptible. This may be due to the fact that the fetus furnishes a different type of tissue marked by the two properties of comparative immaturity and rapidity of cellular growth. The principle of virus increase in rapidly growing cells may be thought of as the reverse of the property of viruses to stimulate host cell growth, as pointed out by Rivers<sup>9</sup> in connection with infectious myxomatosis of rabbits, and as observed to be an essential result of viruses inducing tumor growths.

The microscopic examination of affected tissues, so far as it has been carried, does not disclose a marked cellular reaction. It might be supposed that fetal susceptibility to vaccinia depends on this relative lack of cellular response. That the fetus reacts to some infectious agents with the proliferation and infiltration of inflamma-



tory cells is illustrated by the descriptions of the response of the fetus to the tubercle bacillus<sup>10</sup> and to the submaxillary gland virus.<sup>11</sup> It thus appears that the infectivity of the fetus does not depend on the absence of demonstrable cellular defense. Susceptibility is based on some other type of cellular reactivity, as in postnatal animals, and the relative susceptibility in this case is apparently referable to the properties of fetal cells already mentioned. On the other hand, the lack of universal susceptibility, as pointed out by Woolpert,<sup>8</sup> furnishes a situation equally inexplicable.

Most fetuses were inoculated intracerebrally. The virus content of brain tissue, however, was found to be low at the termination of the experimental periods, much lower in fact than in distant organs. The kidneys, and to a less degree the lungs, were sites of predominant virus localization. The predilection of the skin for pock development is significant in view of the dermal nature of the virus. Judging by the wide distribution of lesions and by the high virus titer of tissues distant from the site of injection, it appears that the fetal guinea pig is parasitized by vaccinia virus in a general way, much like generalized vaccinia in the rabbit and in the chick embryo in the shell, as described by Goodpasture and Buddingh.<sup>12</sup> The virus content of tissues was not dependent, however, on its supply of infected blood, since the blood contained demonstrable virus only twice in ten specimens and then only in low dilutions.

Necrosis was the chief pathological finding. Areas involved were mainly circumscribed with, in the skin, the development of a definite pock. Superficial lesions in the viscera were likewise sharply outlined, except in the kidney where blanched areas indicated a more diffuse process. Hemorrhage occurred frequently in the center of necrotic lesions and sometimes constituted the main gross alteration in the lungs. The lesion in the fetus reached a peak macroscopically in 3 to 5 days and did not seem to progress appreciably during the remainder of the experimental period. A distinct disadvantage in this work was the inability to observe the development of lesions.

The use of vaccine virus propagated in tissue culture and in the chick embryo in the shell has been proposed for human vaccination purposes. Virus of fetal guinea pig origin might be useful to the same end because of its bacterial sterility and high potency, but much must yet be done to standardize and regulate its production.

It should be pointed out that we were unable, by repeated fetal passage, to modify the relation of the virus toward the postnatal guinea pig, on the one hand, and toward the rabbit, on the other. After as many as nine transfers from fetus to fetus, the virus still was ineffective when injected into young or adult guinea pigs by any route and yet retained its ability to induce typical lesions in the rabbit by skin or testicular inoculations. This points to the stability of the virus under these conditions, a property that has been commented on by Goodpasture and Buddingh.<sup>12</sup>

### SUMMARY AND CONCLUSIONS

The vaccine virus was successfully propagated in the fetal guinea pig. The effect of the virus was to induce typical lesions in various organs and tissues. The same virus strain caused only the slightest reaction in the postnatal animal. These results indicate a markedly greater susceptibility of fetal tissues for an infectious agent. The cultivation by other workers of certain viruses in embryonic tissues, when adults of the same species were not reactive, points to the same phenomenon. The mechanism of this comparative effect is not yet clear, although general immaturity and rapidity of cell growth in the fetus are suggested as possible factors.

Lesions were found irregularly distributed in the principal organs, including the skin, and were most common in the lungs and kidneys. Of all the fetal tissues the kidney yielded virus most constantly and in the highest titer, usually measuring 1:10,000.

The vaccine virus was passed through a series of fetuses and was not appreciably modified in its cutaneous activity in the rabbit or in its failure to induce effects in the postnatal guinea pig.

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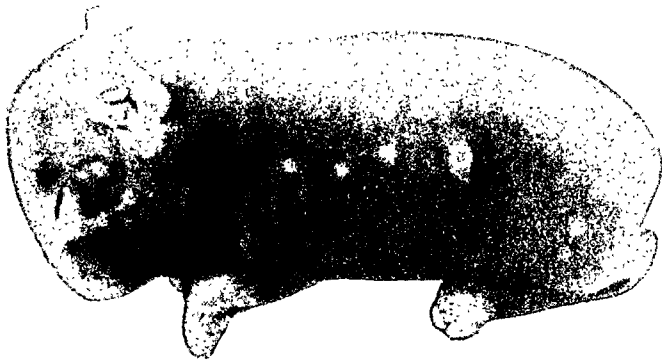
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## DESCRIPTION OF PLATE

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### PLATE 19

- FIG. 1. Fetus delivered dead by cesarean section 6 days after intracerebral inoculation with 0.1 cc. of 1:10 dilution of tissue culture vaccine virus. Multiple, circumscribed cutaneous lesions, some pitted. Patches of adherent fetal membrane around left eye and over scalp.  $\times 1$ .
- FIG. 2. Fetus delivered alive by cesarean section 10 days after intracerebral inoculation with 0.1 cc. of 1:10 dilution of rabbit testicular vaccine (titer 1:1000). Round gray lesions on eyelid, back, side, heel (not in focus), placenta and in adhesion of fetal membranes. Fetus still enclosed in amniotic sac.  $\times 1$ .
- FIG. 3. Lung of fetus delivered alive by cesarean section 7 days after intracerebral inoculation with 0.1 cc. of 1:100 dilution of rabbit testicular vaccine (titer 1:10,000). Diffuse gray areas with hemorrhagic centers on diaphragmatic surface.  $\times 2$ .
- FIG. 4. Lung of fetus of Fig. 2, showing two gray lesions on costal surface.  $\times 1.5$ .
- FIG. 5. Skin lesion of fetus of Fig. 2, in patch of white hair.  $\times 1.5$ .
- FIG. 6. Diffuse necrotic areas in upper pole of left kidney and in both poles of right kidney of fetus of Fig. 2, with hemorrhagic centers in lesions of both upper poles. (White areas are recognized as bits of fetal fat.)  $\times 1.5$ .
- FIG. 7. Two lobes of liver (others removed) of fetus of Fig. 2, showing ten minute gray lesions on surface.  $\times 1.5$ .



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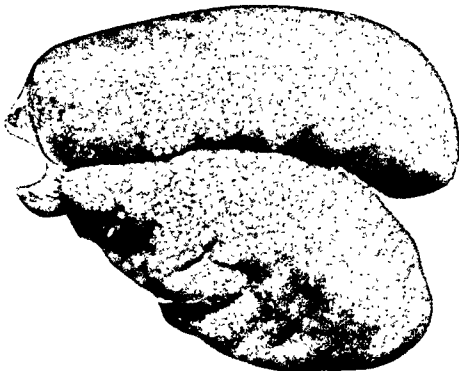
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# SUSCEPTIBILITY OF THE GUINEA PIG FETUS TO THE SUBMAXILLARY GLAND VIRUS OF GUINEA PIGS\*

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## INTRODUCTION

Experimental work with the submaxillary gland virus, in the absence of other susceptible animal species, is limited to the natural host. Since proportions varying from 30 to 84 per cent of stock animals have been found infected, and therefore immune, it has been necessary to study the virus in young animals 2 to 3 weeks old. Guinea pigs of this age are seldom spontaneously infected, but in our study of brain to brain transmission of the virus<sup>1</sup> we found considerable variation in susceptibility, even among these young animals. We have since determined that newborn guinea pigs 1 or 2 days old respond more uniformly to intracerebral injections of the virus. The development of the technique of fetal inoculation in this laboratory by Woolpert<sup>2</sup> placed at our disposal a new method for studying the virus, which practically excludes the possibility of interference from spontaneous infections. The present paper deals with the submaxillary gland virus infection in the fetus and the factors and influences associated with it.

## EXPERIMENTAL

### *Methods and Materials*

The virus used for fetal inoculations was obtained from the salivary glands of guinea pigs supplied by local dealers or of stock animals artificially infected by ourselves. Virus emulsions for fetal injections were prepared in the following manner: the submaxillary glands were aseptically removed from 3 or more adult guinea pigs and ground in a mortar with sterile sand. The pulp was taken up in saline solution to the amount of 1 cc. for each gland. Gross particles were allowed to settle out or were thrown down by light

\* Received for publication September 30, 1935.

centrifugation. The supernatant fluid was considered a stock emulsion for purposes of making dilutions.

The fetuses of pregnant guinea pigs in the 4th or 5th weeks of gestation were most suitable for inoculation. Injections were made intracerebrally or intraplacentally in doses of 0.1 cc.

## RESULTS AND PATHOLOGY

The present paper is based upon a study of the tissues of 60 fetuses of 20 mothers. Experiments were terminated at intervals varying from 3 to 21 days after inoculation. When a litter was composed of 3 or more fetuses, 1 of them was usually left uninoculated as a control. In addition to the various fetal tissues, the submaxillary glands, kidneys, spleen and sometimes the lungs of the mother were studied histologically.

In fetuses that were delivered by cesarean section before they succumbed to the infection, the gross changes generally consisted of petechial hemorrhages in the subcutaneous tissues and viscera, discrete necrotic foci in the liver, and enlargement and infarction of the placenta. Death of the fetus *in utero* was followed within 24 hours by generalized edema and subsequent dehydration. The latter process ultimately reduced the fetus to a blackened, wax-like mass. The placenta in such cases was usually diffusely necrotic, but on two occasions we found it to persist in a fairly healthy condition when the fetus was partially resorbed.

In general, it may be said that the course of the infection in the fetus is governed by two reciprocal factors: the interval elapsing after inoculation and the potency of the inoculum. The influence of the latter factor on the gross pathology in the fetus may be illustrated by the following results of a titration of virus in a gland emulsion. Dilutions of 1:10, 1:100 and 1:1000 were prepared in the manner described above and injected cerebrally into the 3 fetuses of 1 guinea pig. On the 8th day after inoculation the mother was anesthetized and the inoculated animals delivered by cesarean section. The fetus receiving the 1:10 dilution weighed 6.5 gm. and was shrunken, black, and of a wax-like consistence. It had been dead approximately 5 days and its placenta was pale and necrotic throughout. The fetus injected with the 1:100 dilution weighed 12 gm. and was pale, soft and somewhat dehydrated. The placenta contained

both bland and hemorrhagic infarcts, but about half of the tissue was normal in color and texture. This animal had been dead approximately 3 days. The third fetus which had received the 1:1000 dilution weighed 16 gm. and was swollen and gelatinous in consistence and the tissues were blood-tinged. Numerous petechial hemorrhages were present in the subcutaneous tissues and viscera. The placenta was somewhat enlarged but otherwise normal in appearance. This fetus had probably died less than 24 hours before delivery.

It will be noted that the degree of change in each fetus was proportional to the dilution of the inoculum it received and to the length of time it survived after inoculation. In our experience this proportionality was generally very regular when potent virus emulsions were titrated. When weak suspensions were tested the gross changes were much less obvious, but histological examination of the fetal tissues revealed a series of changes that enabled one to reconstruct the course of events leading to the death of the fetus.

So far as we are aware, no other known disease of the guinea pig is accompanied by the presence of intranuclear and cytoplasmic inclusions which might in any way be confused with those of the submaxillary gland virus. Negri, Guarnieri and Bollinger bodies have long been considered important differential and diagnostic aids. In the same way, we have adopted the presence of characteristic intranuclear inclusions in the mononuclear cells as a histological criterion of infection in the fetus. These distinctive cells are associated with all of the lesions in the fetus and are therefore invaluable in studying the course of the infection and assessing the significance of tissue alterations.

Intracerebral introduction of the virus in the fetus was regularly followed by a mononuclear meningitis (Fig. 1), and in severe infections by hemorrhage and liquefaction of the brain. Although the meningitic reaction is most intense about the larger vessels, particularly those of the longitudinal fissure and the ventral surface of the brain, most of the inflammatory cells appear to be of local mesenchymal origin rather than an infiltration of blood-borne mononuclears. Large eosinophilic inclusions are in the nuclei of many of the reacting cells. Unlike the infection in the newborn guinea pig in which the lesions are primarily confined to the meninges, extensive changes are usually found in all the fetal viscera and placenta. In



the liver two kinds of lesions are found: necrotic foci in the parenchyma and perivascular infiltrations and proliferation in the portal triads (Fig. 3). The necrotic foci vary considerably in size and number and do not occur in the absence of the periportal lesions. The intensity of the latter change appears to vary with the extent of the infection in the meninges and placenta. The infiltrating cells consist of myelocytes and non-granular leukocytes. Inclusions are not found in the former cell type, but the mononuclears do become infected and when present in sufficient numbers form a necrotic focus which involves the vascular endothelium.

Following cerebral introduction of the virus, its localization in the placenta is accompanied by proliferation of the local mesenchyme, and in severe infections by thromboses of both the maternal and fetal blood channels. The greatest number of inclusion-containing cells is usually seen in the placental labyrinth (Fig. 2). Perivascular reactions sometimes progress to the point of obliterating the smaller fetal vessels. Where such affected vessels are embedded in the chorionic syncytium and close to the free border bathed by maternal blood, leukocytes from the maternal blood sinus penetrate the epithelium and invade the infected fetal mesenchyme. In some instances a break in the placental barrier was accompanied by thrombosis of the maternal sinus. The size, number and location of the infarctions caused in this manner, and the extent of the vascular disorganization and damage in the liver, seem to determine the survival of the fetus. The remarkable resistance of the chorionic epithelium to infection and injury when surrounded by masses of infected fetal mesenchyme is a striking feature of the placental pathology.

When virus is injected into the placenta instead of intracerebrally, changes occur in both the liver and the placenta similar to those described above. The injected placenta may be double the weight of that of an uninoculated litter mate. This increase in weight is largely due to the infection and proliferation of the placental mesenchyme. It is of interest that "the dome of the central excavation," which is of mesodermal origin, has never been found infected.

In addition to the lesions in the meninges, liver and placenta, small foci usually associated with recent capillary hemorrhage were found in the brain, lungs, spleen, thymus and in the subcutaneous tissues. A number of infected macrophages were once found in a slight reaction in the subcutis overlying an erupting tooth. Inclusion bodies

were always confined to the mesenchymal cells or to tissues derived from the mesoderm; in no instance were they found in epithelial or nerve cells.

### IMMUNITY

It might be supposed that the presence of spontaneous infection in the salivary glands of the mother and her consequent immunity would alter the susceptibility of the fetus or the course of the infection in it. In spite of the fact that the placenta of the guinea pig is of the hemo-chorial type (Grosser's classification) and thus affords optimum conditions for the passive transfer of humoral antibodies, we have found no evidence that the immune state of the mother affected the susceptibility of the fetus or the course of its infection. The glands of 12 mothers whose fetuses were artificially infected were examined histologically and 10 of them contained evidence of long-standing infection. It is of interest in this connection to compare the susceptibility of the prenatal with that of the postnatal animal. Young guinea pigs varying in age from 12 hours to 2 weeks succumbed to intracerebral injections regardless of whether their mothers were spontaneously infected, or whether the young were allowed to suckle immune mothers. It appears from these observations that passive transfer of antibodies, either prenatally, or postnatally with the milk, does not occur in appreciable amounts. These findings are in accord with those of Kuttner<sup>3</sup> who was unable to satisfy herself as to the presence of neutralizing antibodies for this virus in the serum of immune adults.

Further evidence of the inferior or transient nature of the immunity associated with submaxillary gland virus infections was furnished by another type of observation. In this instance the fetuses were inoculated intracerebrally. The infection metastasized to the placenta where maternal leukocytes infiltrated and broke down the placental barrier. Infected fetal macrophages were seen in the maternal blood sinuses. The renal epithelium of the mother was extensively and recently infected, as indicated by the presence of inclusions in the epithelial cells and the absence of cellular infiltrations about the affected tubules. Examination of the mother's salivary glands revealed a single focus of infection which was heavily infiltrated with lymphocytes and in which the inclusions stained very poorly. Cells with inclusions in them have been observed in the lungs of other

mothers when there have been breaks in the chorionic epithelium and inclusion-laden cells in the maternal blood sinuses. In these mothers, however, there has been no evidence of superinfection if their salivary glands contained recent or active lesions.

### DISCUSSION

It is necessary to recognize two important factors in comparing the susceptibility of the fetus with that of the newborn and young guinea pig to the salivary gland virus. The first of these is the ability of the fetus to survive when the brain is partially or totally destroyed. As long as its placenta and cardiovascular system are relatively intact, it can withstand almost unlimited insult; this is not true of the postnatal animal. Thus the susceptibility of the two animals cannot justly be based solely on the period of survival after inoculation.

The second important factor is the greater functional development of the defensive mechanism, as regards the submaxillary gland virus, in the young guinea pig as compared with that of the half-term fetus. Actual details of the development and operation of this mechanism are lacking, but it appears that humoral antibodies are not involved to any appreciable degree. However, the marked cellular response to intracerebral inoculation in the young guinea pig is in sharp contrast to that in the fetus, and may be significant. In the former animal intracerebral inoculation of virus is followed by a considerable exudate of lymphocytes, as well as by infection of the local mesenchymal elements. The lymphocytes do not contain inclusions and inclusion-laden mononuclears are never seen in the blood vessels, although the salivary glands may become infected when the incubation period lengthens to 15 or 18 days — an indication that virus does escape from the central nervous system. In the fetus, on the other hand, there is almost no cellular exudate in the meninges even after cerebral inoculation, and infected mononuclear cells may be seen in the blood vessels and tissues in numerous parts of the body.

The difference in the extent of the infection in the prenatal and postnatal animal does not seem to be adequately accounted for by a difference in tissue susceptibility, for it has been shown by Cole and Kuttner<sup>4</sup> that when virus is injected into such sites as the lung, the testes or the tongue of young guinea pigs, inclusions are formed in

the local mesenchymal cells. It is problematical whether the presence or absence of a meningeal exudate is the important limiting factor or whether the difference in tissue susceptibility is one of degree rather than of category. However, it may be said that as a test animal for the presence of the virus the fetus is superior to the postnatal animal. Amounts of virus that cause not so much as a rise in temperature in young guinea pigs will, in the fetus, proliferate and ultimately bring about its death. In addition, when large amounts of virus are required, placental inoculations produce a far richer supply in fetal tissues than can be obtained from postnatal guinea pigs, either spontaneously or artificially infected.

### SUMMARY AND CONCLUSIONS

Infection of the guinea pig fetus with submaxillary gland virus is described.

Typical inclusion bodies were found and were taken as an indication of the presence of the virus. On this basis the virus was apparently generalized throughout the fetus. Lesions appeared in most of the organs, especially the brain, liver and placenta. When death occurred, it seemed to be due to interference with the circulation by lesions in the placenta and liver.

It was determined that the fetus is better suited in many respects for experimental work with the submaxillary gland virus than the newborn or young guinea pig. Interference from spontaneous infections is avoided, smaller amounts of virus can be detected and the yield of virus is greater.

The susceptibility of the fetus is not altered by the immune state of the mother.

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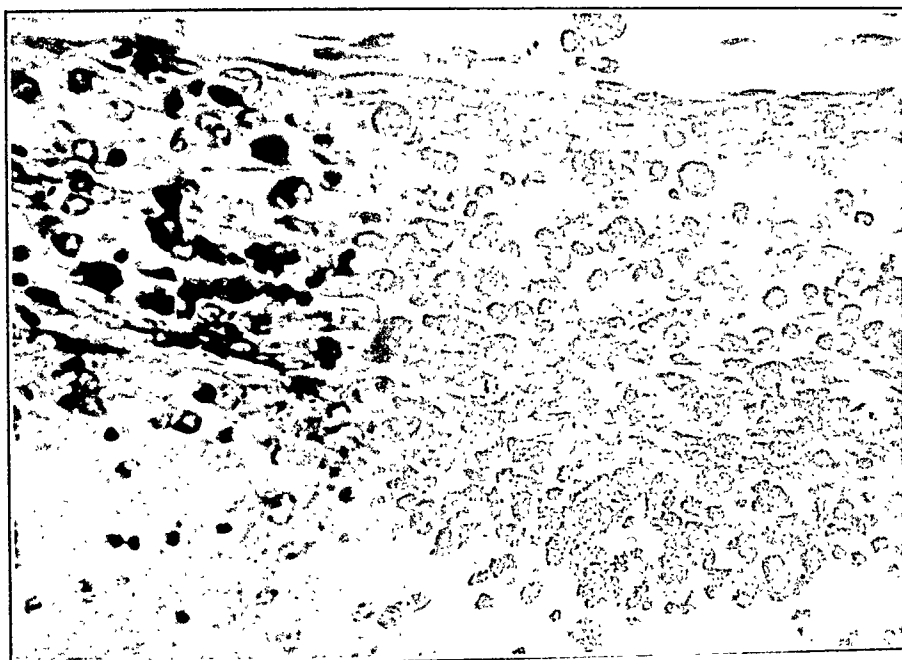
## DESCRIPTION OF PLATE

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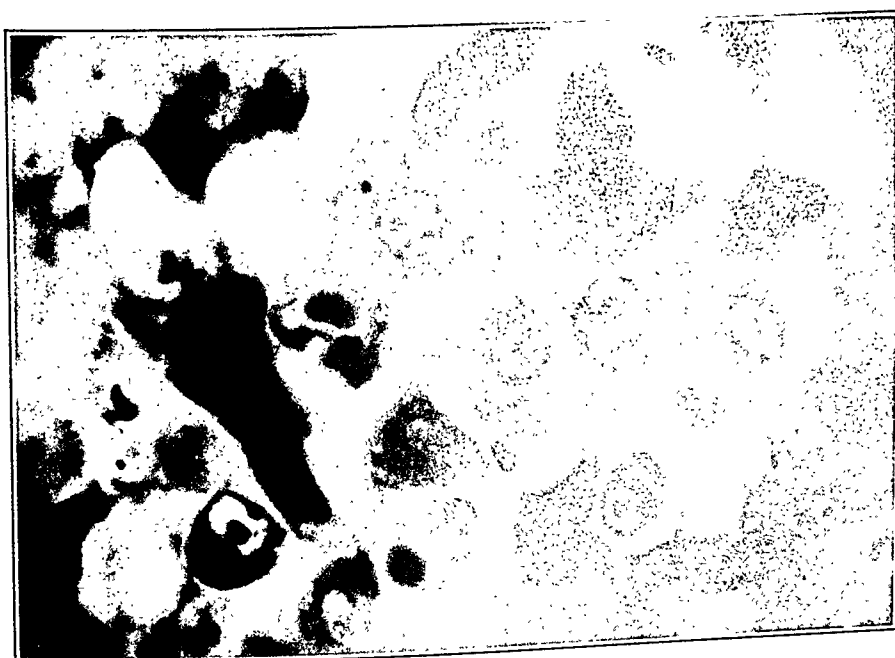
### PLATE 20

Photomicrographs of paraffin sections stained with eosin-azure. Wratten-Wainright green filter "B" employed.

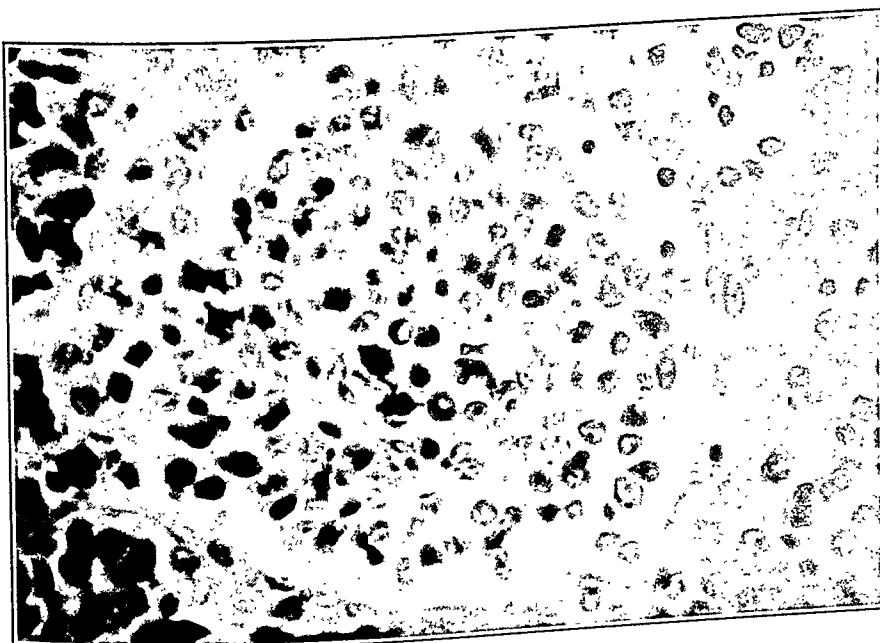
- FIG. 1. Meningeal reaction following intracerebral inoculation of submaxillary gland virus in a fetus 5 weeks old. Slight involvement of the superficial cortex and extravasation of erythrocytes are shown.  $\times 250$ .
- FIG. 2. Placental infection following intracerebral inoculation of virus. Several inclusion-laden mesenchymal cells are shown in the fetal labyrinth.  $\times 1000$ .
- FIG. 3. Perivascular reaction in portal area of fetal liver following intracerebral injection of virus.  $\times 250$ .



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## PERICARDIAL LESIONS IN RHEUMATIC FEVER \*

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The tendency to affect serous membranes is one of the most characteristic features of rheumatic infection. Because of the striking appearance of pericardial inflammation in rheumatic fever its presence was frequently noted by early writers, who stressed its significance as evidence of cardiac disease. In 1761 Pulteney<sup>1</sup> reported a case of adherent pericardium in a young man who had suffered from fever and arthritis 2 years earlier and who died with signs of heart failure. Baillie<sup>2</sup> (1793) first described the acute form of pericarditis, with exudation of "coagulable lymph" from blood vessels in the pericardium and fluid in the pericardial cavity. He also recognized the thickening, congestion and adhesive tendency of the membrane, the conversion of the pericardium into cartilage, and the fact that pericarditis could be survived. A completely adherent pericardium in a 14 year old girl who died of acute rheumatic fever was reported by Wagstaffe<sup>3</sup> in 1803. Similar cases were reported by Crowfoot<sup>4</sup> and by Wells.<sup>5</sup> The former described 1 case with ossification of the adherent pericardium. The data on the case, however, are insufficient to establish a rheumatic etiology of the lesion.

By the beginning of the nineteenth century textbook writers were describing gross pericardial lesions as lucidly and accurately as to-day. The reticulated, curdled appearance of fibrinous pericarditis was likened by Corvisart<sup>6</sup> to the internal surface of the second stomach of a calf, and by Laennec<sup>7</sup> to "bread and butter," an expression retained by Hope<sup>8</sup> and all textbook writers after him. Laennec outlined the sequence of events in pericarditis as beginning with redness of the pericardium in isolated plaques, continuing with an albuminous exudate covering the entire surface, and concluding with healing and the resulting pericardial adhesions. He either did not recognize or did not stress the etiological significance of acute rheumatic fever.

\* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.  
Received for publication October 7, 1935.



Although the etiological relation between pericarditis and rheumatic fever had already been noted in isolated cases, credit is generally given to Bouillaud (1840) <sup>9</sup> for conclusively demonstrating clinically and pathologically, in a large series of cases, the constant association of pericarditis, endocarditis and rheumatic fever. Yet in 1808 Dundas,<sup>10</sup> in 9 cases, had recognized pericarditis as occurring at the beginning or termination of one or more attacks of rheumatic fever. Of the 6 cases in which there was a postmortem examination, 1 had a pericardial effusion and the others an adherent pericardium. Likewise, Andral <sup>11</sup> in 1826 fully recognized the relation of 4 cases of pericarditis to acute rheumatic fever. Hope <sup>8</sup> in 1832 pointed out the importance of the latter as a cause of both acute and chronic pericarditis. He described the characteristic shaggy, flocculent, rough-patterned gross appearance of pericarditis and analyzed the three essential features of the acute disease as unusual redness of the membrane, "coagulable lymph" adhering to the surface and fluid effused into the cavity. He realized that the frequently resulting adherent pericardium, although a healing process, produced organic disease which might be fatal.

Very little has since been added to these gross descriptions of rheumatic pericarditis. The earliest studies of the microscopic appearance of pericardial lesions were made in 1890 by Krehl,<sup>12</sup> who in 8 cases of pericarditis associated with valvular heart disease described round cell infiltrations, especially about the smaller blood vessels, and intimal sclerotic changes in the larger blood vessels. But since some of these cases were undoubtedly not of rheumatic etiology and most of them patients in the fifth decade of life or beyond, the significance of these findings is somewhat lessened. More detailed and accurate microscopic descriptions followed the recognition of the specific Aschoff nodules as a rigid pathological criterion of rheumatic heart disease. Coombs<sup>13</sup> was the first to note them in the pericardium in 4 cases of acute rheumatic fever. They occurred only in the visceral pericardium, singly or in groups, and, perhaps because of freer surroundings, more loosely arranged than the Aschoff bodies in other cardiac tissues. Wätjen<sup>14</sup> described a case with Aschoff bodies in the walls of the small branches of the coronary artery. Mönckeberg<sup>15</sup> reported 2 cases of rheumatic pericarditis with Aschoff bodies in the pericardium, in 1 of which they were macroscopically visible as grayish, tubercle-like nodules over the right

ventricle and auricle. Sacks<sup>16</sup> also observed that they were often quite large and were found most frequently during the period of organization of the pericarditis.

More recent descriptions have included the microscopic appearance of the acute exudative phenomena. Coombs<sup>13</sup> noted endothelial proliferation, degeneration and desquamation. The raw surface was covered with fibrin. New capillaries and cells in the visceral pericardium, uniting with similar structures in the parietal layer, formed adhesions. Below the endothelium there was a zone of leukocytosis, chiefly mononuclear, and a layer of new connective tissue and capillaries. In a similar description by Bronson, Carr and Perkins,<sup>17</sup> grossly there was fusion of the thickened parietal and visceral layers, readily distinguishable and bound together by a third central layer of edematous connective tissue. Microscopically the outer parietal layer was composed of dense hyaline connective tissue covered superficially by a looser vascular fatty tissue, the inner or visceral layer of loose areolar tissue with many strands of compact hyaline connective tissue injected with small blood vessels, and the third central layer of edematous granulation tissue with occasional irregular masses of fibrin. Throughout, but particularly in the central and visceral layers, there was a marked infiltration of leukocytes, with a predominance of lymphocytic and plasma cells. In the central layers there were numerous polymorphonuclear leukocytes and endothelial cells, some of which were multinucleated.

Swift<sup>18</sup> emphasized the similarity in reaction of the pericardium and of other tissues in rheumatic disease. He stressed the difference between the non-specific extensive serofibrinous reaction, which was not significantly different from the response of other lining membranes to extensive injury, and the more localized granulomatous rheumatic lesions that represented a specific response to a rheumatic infection.

Sacks<sup>16</sup> considered fibrinous pericarditis one of the most distinctive lesions of rheumatic fever. He described the process as beginning with fibrin deposition followed by serous effusion and the exudation of leukocytes and erythrocytes. There was also desquamation of serosal cells. These, however, persisted below the fibrin for some time. The subpericardial tissues were edematous, containing numerous capillaries and fibroblasts and there was a diffuse cellular reaction with concentration around the small vessels. The blood

vessels showed endothelial swelling and proliferation, sometimes with thrombus formation. In a careful study of 250 serial sections from the heart of a case of acute rheumatic fever Darré and Albot<sup>19</sup> distinguished the fibrinous exudate on the surface, the reaction of the pericardial serosa proper and that of the subepicardial fatty tissue. Recently, Klinge<sup>20</sup> emphasized the similarity of rheumatic pericarditis to the rheumatic reaction of other cardiac structures and pointed out that the first stage was not the deposition of fibrin on the surface, but the same type of "fibrinoid" swelling of the ground substance of connective tissue as occurs elsewhere in the heart.

Peculiar polypoid and cyst-like formations were found by Lauche<sup>21</sup> in a case where they were probably due to desquamated epithelium persisting in the pericardial sac, fused at their tips and enclosing hemorrhagic exudate, and by Bohrod<sup>22</sup> in a case where, though more solid, they probably represented similar cysts whose original fluid content had become organized.

As the above review indicates, previous descriptions of the microscopic features and sequence of events have been presented in isolated cases, or as incidental findings in more detailed studies on other cardiac structures. We have, therefore, undertaken a detailed and systematic investigation of the pericardial lesions in a large series of cases of rheumatic heart disease which came to postmortem examination.

#### MATERIAL AND METHODS

There were 87 cases ranging from 17 months to 67 years of age. Of these, 68 presented myocardial Aschoff bodies and other evidences of active rheumatic infection (Rothschild, Kugel and Gross<sup>23</sup>). The remaining 19 presented the characteristic valvular deformities and old auricular lesions of inactive rheumatic fever. No Aschoff bodies were present in these inactive rheumatic cases. The first mentioned 68 cases were segregated into four groups according to the classification outlined by Gross and Ehrlich:<sup>24</sup>

GROUP I. Active cases in which the first attack was fatal (10 cases).

GROUP II. Active cases in which there was one attack prior to the final fatal recurrence (18 cases).

GROUP III. Active cases with repeated attacks and death during an acute recurrence (12 cases).

GROUP IV. Active cases in which death occurred with signs of congestive heart failure but without clinical evidence of an acute rheumatic recurrence (28 cases).

The remaining 19 cases of clinically and pathologically inactive rheumatic fever will be referred to as Group V. The purpose of this classification is to correlate the pathological findings with the clinical course of the disease.

The normal histology of the pericardium was studied in 35 additional cases evenly distributed between the ages of 3 months and 74 years, without clinical or pathological evidence of cardiac disease. Standard sections of both normal and abnormal pericardiums were made according to the technique described by Gross, Antopol and Sacks.<sup>25</sup> The detailed methods of fixation and the stains employed have been described elsewhere.<sup>26</sup> After fixation and cutting, the parietal layer of pericardium is not visible on microscopic section except where it has become adherent to the visceral layer because of inflammation. Our microscopic descriptions of both the normal and abnormal pericardium, therefore, will generally refer to its visceral layer or epicardium.

### GROSS PERICARDIAL LESIONS

While the gross changes in rheumatic pericarditis have been adequately described before, a few comments as to the incidence, nature and distribution in the larger series of cases which we have studied appear pertinent, particularly with reference to the above clinical classification.

In general, there was a much higher incidence of gross pericarditis in the cases where death appeared clinically to have taken place in an attack of acute rheumatic fever, *i.e.* in the first three groups. Of the 10 cases in Group I in which death presumably occurred in the first attack, 6 had definite gross pericarditis consisting of a fibrinous exudate over the entire pericardium with thickening, glazing and adhesions. In 3 of these there was also a variable amount of straw-colored turbid fluid containing many shreds of fibrin. In 1 of these the exudate was definitely fibrinopurulent. A 7th case showed a localized patch of fibrous thickening over the right auricle.\* The remaining 3 appeared to have a normal pericardium.

\* The so-called "milk patches" were disregarded because of their uncertain and variable etiology.

In Group II, where one attack preceded the final one, there was an even higher incidence of gross pericarditis. Fourteen of the 18 cases (78 per cent) showed a universal pericarditis, consisting generally of the thick, shaggy, bread-and-butter type of lesion with numerous fibrous adhesions. Seven of the 14 had a completely adherent pericardium with obliteration of the pericardial sac and occasional adhesion to neighboring extracardiac structures; 4 of them had a serofibrinous or serohemorrhagic exudate partially sacculated between the adhesions. Of the remaining 4 cases in Group II, 3 revealed localized fibrinous and fibrous lesions over the auricles or auricular appendages, and only 1 had a grossly normal pericardium.

In Group III (recurrent attacks of acute rheumatic fever) all of the 12 cases showed some evidence of gross pericarditis. In 7 in which it was universal, 6 had complete obliteration of the pericardial cavity, and the 7th, the youngest patient in the group (4½ years), had a serous effusion with fibrinous exudate and easily separated adhesions. In the remaining 5 cases the lesions were localized in isolated areas of the pericardium.

Group IV, comprising 28 cases where death had apparently occurred of cardiac decompensation but where myocardial Aschoff bodies were present on histological examination, included 4 cases with gross universal pericarditis and 6 with local pericarditis in the form of isolated adhesions or patches of thickened pericardium. The remaining 18 were grossly normal. In 3 of the cases with universal pericarditis the lesions consisted of fibrous adhesions with thickening (in 1 there was a localized calcareous mass in the pericardium), and in the 4th there was a serosanguineous exudate with fibrinous deposits and adhesions which could be easily separated. Two of the cases with localized pericarditis likewise showed a serofibrinous exudate.

In Group V, comprising 19 cases with chronic, rheumatic valvular disease but without Aschoff bodies in the myocardium, 4 had a universal pericarditis consisting of an adherent pericardium with obliteration of the pericardial sac, 5 showed localized fibrous or fibrinous pericardial thickenings and 10 were grossly normal.

The purest form of acute pericardial lesions can be studied best in Group I where there was but one attack of rheumatic infection. Although the attack was severe enough to end fatally in these cases, the

local pericardial infection was either too mild or of too brief duration to give gross lesions in 3 of the 10 cases, and caused only local lesions in a 4th case. In the other 6 cases with generalized pericardial inflammation, a fibrinous pericarditis was visible, and despite the brief duration of the process (as early as  $3\frac{1}{2}$  weeks in some cases), there was already distinct evidence of healing in the form of grossly visible adhesions. Furthermore, half of these 6 cases presented a significant pericardial effusion.

In Groups II and III, in which there was more than one attack of rheumatic fever and where death occurred during an acute exacerbation, only 1 case appeared normal on macroscopic examination. These groups, in contrast to Group I, presented a more advanced state of healing with scar formation. In half of the cases adhesion between the pericardial layers was so extensive as to completely obliterate the pericardial cavity. In the remaining cases the pericardium was thicker than in Group I, and the fibrinous exudate much more extensive and showed more advanced organization.

The infrequency of generalized pericardial involvement in Groups IV and V can be correlated with the absence or mildness of clinical rheumatic manifestations in these cases. In spite of the fact that there was definite pathological evidence that such rheumatic infection had not only occurred in the past but, as in Group IV, had been active at the time of death, the customary exudative phenomena in the serous membranes (pericardium, synovia, and so on) may have been extremely mild.

In all five groups when a localized pericarditis was present there seemed to be a tendency for this to occur more frequently over the posterior wall of the left auricle. This area, therefore, should be carefully examined for such lesions. These may be rather insignificant and easily overlooked, particularly as they are apt to form inconspicuous adhesions at the cephalad portion of the pericardial fold. It is possible that this left auricular localization of the pericardial process may represent a contiguity spread of an auricular endocardial lesion.

This site of predilection for localized lesions and sacculated effusions, together with the occasional presence of an isolated fibrous tag on the posterior wall of the ventricular chambers near the apical region, was pointed out to the authors by Dr. Emanuel Libman.<sup>27</sup>

## HISTOLOGY OF THE NORMAL VISCERAL PERICARDIUM

The visceral pericardium (Fig. 1) is composed of three layers: superficially, the epithelial layer; below this, and frequently separated from it by a loose reticular zone of varying thickness, is the fibrous or fibroelastic lamina propria; between the latter and the myocardium there is an adipose layer containing numerous nerves, blood vessels and lymphatics. The epithelial layer consists of a single row of mesothelial cells, generally not as flat as those lining other serous membranes, but varying in shape according to their location and the phase of the cardiac cycle. Usually they are cuboidal, their margins quite distinct, the protoplasm finely granular and the nuclei oval or round and staining deeply. In routine sections these cells are not always clearly visible, but the surface of the pericardium appears bound by a fine line (membrane) with occasional flat nuclei, in distinction to the raw appearance resulting when these cells have been desquamated by injury.

The structure of the lamina propria, a rather loose fibroelastic layer of variable thickness, is somewhat dependent on the age of the individual but its variations are not as marked as in other portions of the heart, such as blood vessels, auricle, and so on. At 3 months it is quite slender and consists of a framework of loose interlacing areolar tissue containing small irregular whorls of dense collagen. There are also present comparatively few, loosely arranged, fine, wavy elastic fibers. These are more conspicuous where elastic reinforcements are received from the moderately elastified adventitia of the pericardial vessels. At this early age the lamina propria is not yet definitely separated from the other two layers as there is comparatively little adipose tissue and the collagenous tissue is intermingled with the fat cells.

A clearer distinction into the three layers is visible in the second year. At this time the fibroelastic structure of the lamina propria appears thicker and more clearly demarcated, sometimes separated from the epithelium by an acellular loose zone of reticular tissue, and from the myocardium by a rather thick layer of adipose tissue. With increasing age the fine, wavy elastic fibers of the lamina propria become much more numerous and tend to form a distinct band on its superficial aspects parallel to the surface. This band is connected by numerous short oblique and perpendicular elastic fibers with sub-

jacent, interlacing collagenous fibers. When the loose reticular zone separating the lamina propria from the epithelial surface becomes appreciably widened, a fine elastic membrane may sometimes be found immediately beneath the latter. At times, the loose reticular zone may contain smooth muscle fibers. From the fourth decade on, the elastic fibers of the dense collagenous lamina propria are thicker and often form a rather conspicuous, regularly arranged layer of elastic tissue superficial to the collagenous mass. The latter is irregularly permeated with scattered elastic fibers.

The adipose layer consists of the classical signet ring cells with wavy, circular or oval outline. A fine reticular framework running through this tissue contains occasional arterioles and delicate capillaries at the junction of fatty lobules. There are also present numerous larger vessels in the region of which the ordinarily delicate stroma becomes strengthened by offshoots from the vascular adventitia. Where the adipose layer adjoins the lamina propria there are numerous small vessels which only rarely extend into the latter. Numerous nerves are found, especially in the region of the blood vessels.

Histiocytes were generally present and leukocytes were found occasionally in the various pericardial layers of the hearts that constituted the normal group. The histiocytes appeared in moderate numbers in the lamina propria and more sparsely in the adipose layer. Leukocytes, when present, were invariably of the mononuclear variety, usually small lymphocytes. They were rarely seen in the lamina propria and then only in small numbers. In the adipose layer their presence was somewhat less rare. They were usually observed in small numbers in the vicinity of small blood vessels. These cells probably represent evidences of pericardial irritation occurring either in the course of non-rheumatic infections present in these cases before death, or due to mechanical irritation (rubbing) of an enlarged auricle or ventricle. These cellular collections may bear some resemblance to the mild lesions in Groups IV and V. In the latter, however, the cellular aggregates are more marked and more extensive and associated with inflammatory changes (increased and dilated blood vessels, and so on).

To summarize, the significant features in the normal pericardial structure are the paucity of leukocytes; the regular arrangement of the three layers; the intact epithelium with its single row of cells; the



slender layer of elastica and collagen containing but few fixed connective tissue cells and, rarely, occasional capillaries; and the adipose layer with its fatty cells, blood vessels, nerves and fine reticular stroma.

## MICROSCOPIC FEATURES OF RHEUMATIC PERICARDITIS

### *Epithelial Alterations*

One of the early changes in rheumatic infection of the pericardium involves the lining mesothelial cells which begin to proliferate, become loosened from the underlying structures and are desquamated into the pericardial cavity (Fig. 2). This may be associated with a thickening of the entire layer superficial to the epithelial basement membrane. In many cases the epithelial cells retain their configuration and may be seen lying in rows detached from the remainder of the pericardial membrane. Within a given section of the same case they may be observed in all the various stages mentioned. Particularly where gross pericarditis is absent or largely confined to localized areas, one may observe stretches of intact epithelium occasionally interrupted by sites of uncovered pericardium, or by proliferated and desquamated epithelial cells.

The frequent tendency of the desquamated epithelial cells to preserve their intact cellular structure and become detached in single-layered strips sometimes leads to bizarre pseudoglandular formations (Fig. 3). Several such strips seem to join at their free ends to enclose clear cyst-like spaces which are sometimes already filled with a serous or hemorrhagic fluid. If the fluid portion is absorbed, as in the cases reported by Lauche<sup>21</sup> and Bohrod,<sup>22</sup> solid as well as cystic tumors result, which were not, however, observed grossly in our cases. Occasionally, small polypoid structures are formed by epithelial cells which proliferate without detaching.

Usually, when there is an extensive fibrinous exudate, the epithelium is completely missing except for occasional groups of cells found free in the pericardial exudate. However, sometimes rows of intact cells are seen somewhat below the surface of the thickened epicardium, covered by an organizing fibrin-containing layer of tissue rich in cells and blood vessels. In such cases it appears as if the fibrinous material was exuded between the epithelial cells without disturbing them. Not only fibrinous exudation but certain other

alterations in the subjacent collagen, in the blood vessels or in the fatty layer, will be described as occurring occasionally while the epithelium is still intact. From these and other observations to be mentioned, it appears that while the changes in the pericardial epithelium occur early, they are neither the initial nor the essential feature of rheumatic pericarditis.

### *Changes in the Lamina Propria*

It is generally difficult to determine the earliest stages in the inflammatory process, so obscured are they by the extensive microscopic alterations in the pericardium which have already occurred even when death has supervened comparatively early in the course of a first attack of rheumatic fever. But in the occasional sections in which the earlier and milder lesions can be observed free from later secondary complication, the lamina propria and, more specifically, its collagen undergo the same swelling and degeneration that characterize primary rheumatic lesions elsewhere in the heart. The normally regular, interlacing feltwork of collagen is seen to be swollen, edematous, irregularly frayed and distorted. The entire lamina propria, particularly the portion adjoining the adipose layer and containing the greatest concentration of collagen, becomes infiltrated with numerous inflammatory cells, all or most of which are round cells (Fig. 4). The majority of these are small with scanty cytoplasm and deeply staining nuclei resembling or identical with the lymphocyte, but there are also numerous large round cells with considerable cytoplasm and oval or round vesicular nucleus, and varying numbers of plasma cells. Occasionally, foci of polymorphonuclear leukocytes appear which do not seem to be regular constituents of the rheumatic infiltrate.

In some of these early lesions the degenerated collagen takes on eosinophilic properties beneath an epithelium still intact except possibly for some proliferation and beginning desquamation. At this stage there may still be no fibrinous exudation, but there is usually an increased vascularization especially at the junction of the adipose layer with the lamina propria but also within the latter layer itself. Frequently, the smaller arterioles, venules and capillaries show swelling and proliferation of the endothelium, a phenomenon often characterizing rheumatic vascular lesions elsewhere. When

fibrinous exudation occurs, organization soon follows and the lamina propria can be seen clearly demarcated by a line of elastic tissue from the organizing exudate (Fig. 5).

In some cases, particularly in Group I, conspicuous lesions were seen within the pericardial wedge adjoining the ring of the mitral and tricuspid valves and in the deeper portion of the adipose layer near the myocardium (Fig. 6). These lesions consisted of edema, excessive vascularization, infiltrations with round cells, polymorphonuclear leukocytes, Aschoff bodies and the tendency to early and marked fibrosis. Comparison of these lesions with those in the adjoining ring or subjacent myocardium suggested that the pericardial alterations were due to extension of the inflammatory process from the first mentioned structures. Such pathogenesis, however, did not exclude a simultaneous involvement of the pericardium in its more superficial aspect, as already described, probably through the blood vessels adjoining the lamina propria.

### *Changes in the Adipose Layer*

Very early in the course of the inflammatory process the adipose layer shows pathological alterations which, when well marked, indicate a full blown process involving every portion of the pericardium. The earliest changes appear to be an edema, cellular infiltration and increased vascularization of this layer. Sometimes there is extravasation of red blood cells into the fatty tissues or superficially into the pericardial cavity. The fine reticular stroma forming the framework becomes thickened, irregular and greatly increased in amount. The normal outline of the fat cells becomes distorted and compressed by the surrounding edema and exudate. The inflammatory cells resemble those already described except that while round cells still predominate, there is more often a considerable representation of polymorphonuclear leukocytes. The cellular infiltration tends to form foci around the smaller branches of the pericardial blood vessels.

### *Advanced Pericardial Changes*

The well developed case of rheumatic pericarditis presents a markedly thickened pericardial membrane covered by a dense layer of fibrin containing a few epithelial and inflammatory cells. Below lie the remains of the lamina propria, swollen, edematous, distorted and

sometimes still showing eosinophilic swelling and degeneration. In this area there is an intense inflammatory exudate consisting chiefly of round cells and occasionally of polymorphonuclear leukocytes and nuclear débris. In addition there is marked vascularization of this region which contains numerous small arterioles and capillaries, many of which show endothelial proliferation, swelling and other vascular changes to be described. There is marked tendency for the inflammatory cells to concentrate around these blood vessels. Occasionally, at this stage, an Aschoff body is present. When organization is already visible, as is usually the case, there are in addition numerous fibroblasts and congested capillaries of the characteristic granulation tissue type.

In the adipose layer the normal regular conformation of the fat cells has become obliterated by an albuminous and sometimes fibrinous exudate. There is an increase in the reticular stroma which often forms thick bands running through this layer. Throughout there is an intense vascularization and leukocytic infiltration, especially around the blood vessels. The formation of granulation tissue appears to occur particularly from this layer. At an early stage there is an unusual preponderance of capillaries and arterioles congested with erythrocytes. These vessels often have a corkscrew appearance and run perpendicularly toward the surface. There are numerous fibroblasts and, very early, a marked formation of connective tissue. Where organization is further developed, fibrosis may extend even to the surface and often the remnants of fibrin are visible as masses enclosed within this organizing granulation tissue.

In those cases where recurrent attacks of rheumatic fever occurred there is a definite formation of layers (Fig. 7). The original adipose layer shows marked fibrosis sometimes consisting of a vascular connective tissue with numerous round cells and a frequent increase of collagen. Part of the fatty tissue may still be visible, markedly infiltrated with fibroblasts, congested capillaries and inflammatory cells. Above the adipose layer lies the thickened and generally inflamed lamina propria. This is often separated by a fine distinct line of elastica from the next and most superficial layer. The latter consists of young granulation tissue showing an intense infiltration with round cells and fibroblasts. The surface of this last layer is frequently covered with fibrin already invaded by granulation tissue with irregular patches of complete organization. In a great number of cases in this

group this visceral pericardium with its partially organized fibrinous exudate is undergoing adhesion with the similarly altered parietal layer. Between the two inflamed layers of the pericardium there is an extremely loose granulation tissue consisting of fibroblasts, connective tissue fibers and granulation tissue capillaries. In these cases it is difficult to say whether the deeper fibrotic layers represent the healed inflammatory process of previous attacks or whether all the changes described have occurred within the last attack itself. The latter, if true, would indicate a greater tendency to early fibrosis in cases where previous attacks of rheumatic fever had occurred than in those where the patient succumbed in the first attack.

### *Lesions in Groups IV and V*

Certain mild pericardial changes which differed in some respects from the early lesions described above were especially frequent in the cases in Groups IV and V in which there was no history of rheumatic fever. The essential feature in these cases was the presence of inflammatory cells and blood vessels in the deeper portion of the lamina propria adjoining the adipose layer (Fig. 8). Normally, as we have indicated, the lamina propria is invariably or almost invariably free from blood vessels or cells except for occasional fixed connective tissue cells. In 38 of the 47 cases constituting Groups IV and V, this layer showed infiltrations with small and large round cells largely resembling the inflammatory cells described in the more acute cases of pericarditis. They were, however, less numerous and less extensive than in the acute cases already described. Furthermore, there were to be seen numerous capillaries, arterioles and small arteries, some of which showed alterations like those seen in other cases of rheumatic fever. Sometimes these inflammatory changes were present in one or two of the standard sections and not in any of the others, occasionally being found only in the pericardium which lies behind the aorta or pulmonary artery (Gross<sup>28</sup>).

In 4 of the cases in Group IV, Aschoff bodies were found in the pericardium, in 1 case in the immediate vicinity of a large coronary artery, and in 1 case associated with a banded arrangement of small and large round cells. While the most superficial layer of the pericardium was at times thickened, there were no reduplications such as are seen in old rheumatic auricular lesions. However, a definite

disorganization of the collagenous and elastic structure of the pericardium was often seen. In the adipose layer there was an increased amount of stroma and numerous localized foci of round cells, especially around the generally more numerous blood vessels. In not all of the cases in Groups IV and V were the changes limited to those just mentioned. An occasional case revealed fibrinous exudation. The surface epithelium was usually intact, but there were occasional cases showing epithelial proliferation, pseudogland formations or polypi on the surface. In addition to the above findings some cases showed fibrosis and adhesions, occasionally with increased elastification of the pericardium. In a large majority of cases there was not merely an increase in the number of blood vessels throughout the pericardium but, in addition, other rather characteristic qualitative vascular alterations such as described by Gross, Kugel and Epstein<sup>29</sup> for other portions of the heart.

### *Alterations of Blood Vessels*

Most of the blood vessels seen in acute pericarditis are dilated congested capillaries, often round but more characteristically elongated, tortuous, or of corkscrew shape with the long axis perpendicular to the surface. These were generally components of granulation tissue in the course of healing. Certain other capillaries, apart from granulation tissue, showed in their endothelial linings a striking swelling and proliferation which was occasionally sufficient to form a verrucous projection into the lumen. Rarely, the proliferated endothelial cells became desquamated and degenerated to form a plug which became organized and recanalized. Not infrequently in the active cases small pericardial veins contained thrombi. The small arteries and arterioles in the majority of cases showed changes identical with those described by Gross, Kugel and Epstein.<sup>29</sup> Frequently there was a marked increase of fibrous tissue in the adventitial and peria adventitial region of the blood vessels. In the cases of the first three clinical groups there was not infrequently medial hypertrophy, especially behind the aorta and in the wedge of pericardium near the auriculoventricular junction, and sometimes intimal fibrosis occasionally with increased elastification of the intima. Perhaps the most frequent and characteristic lesion was that which Gross, Kugel and Epstein<sup>29</sup> have termed the intimal musculo-elastic hyperplastic

lesion, a marked development of longitudinally arranged smooth muscle cells which constitute several layers internal to the lamella elastica interna, and an increased elastification of the intima, sometimes with swelling of the endothelial cells. Because of their distinctive appearance and frequent occurrence in rheumatic fever, these lesions, which were found not infrequently in the cases of Groups IV and V, as well as in the more active cases, formed important corroborative evidence of rheumatic infection of the pericardium. Intimal musculo-elastic hyperplastic lesions were especially numerous in the pericardium behind the pulmonary artery and aorta.

### *Aschoff Bodies*

Aschoff bodies apparently identical with those observed in the myocardium, were found in 2 cases of Group II, in 1 case of Group III, in 4 cases of Group IV, but with the greatest frequency in Group I where they were present in 6 of the 10 cases. They were thus present in about one-fifth of the cases in which Aschoff bodies were found elsewhere in the heart. Observed in the pericardium most often in relation to branches of the coronary arteries, occasionally they occurred independently of adjacent blood vessels and sometimes could only be found in the pericardium behind the pulmonary artery or aorta. In the free connective tissue they generally assumed the reticular or mosaic form described by Gross and Ehrlich,<sup>24</sup> but in the proximity of blood vessels, especially of larger size, there was a greater tendency toward the polarized form (Fig. 9).

## DISCUSSION

### *Incidence of Pericarditis in Rheumatic Infection*

A detailed, macroscopic and microscopic study of the pericardium in rheumatic heart disease made by Coombs<sup>30</sup> revealed a progressive diminution of pericardial lesions varying from 100 per cent in patients dying in the first decade to about 25 per cent in those dying in the fourth or subsequent decades, with a total mortality of 53 per cent. According to Coombs the lesser incidence in the later age periods indicated that these patients suffered from milder lesions and therefore survived longer. The average incidence of gross universal pericarditis in the first three groups was 70 per cent, with local pericardial lesions in an additional 20 per cent. In Groups IV and V

there was an average of 17 per cent showing universal pericarditis with local pericarditis in an additional 23 per cent of the cases. Microscopically, however, there was distinct evidence in one or more of the sections of pericardial inflammation in 50 per cent of the



areas, by increased vascularization and structural alterations of the blood vessels. There was an absence of fibrinous exudation or other evidence of acute inflammation. The overlying epithelium in general was intact and there was no formation of adhesions.

The earliest and mildest pericardial lesions in rheumatic fever probably occur in those patients who suffer a single attack of the disease without succumbing. Such cases could be obtained only if immediately following recovery from rheumatic fever the patient succumbed to some unrelated accident. We may surmise that the lesions in these cases would not differ essentially from the early mild lesions found in occasional sections in Group I. The other lesions that have been described for Group I represent the course of the rheumatic infection when this has been sufficiently severe to cause death. On the other hand, it is possible that the mild lesions that were described for Groups IV and V represent the course of the rheumatic infection when the patient survives. In other words, these lesions may be the residual findings from a preceding rheumatic pericarditis of a mild nature. It is difficult to say whether in these cases there once was an acute, exudative fibrinous pericarditis with loss of epithelial lining and with subsequent restoration to complete integrity, or whether the original lesions were so benign that there never was a generalized irritative reaction on the part of the lining membrane. Correlating this with the absence of clinical features of rheumatic fever, one might then assume that in these cases there was a similar absence of exudative phenomena elsewhere in the body (for example, joints) which usually determines the recognition of the clinical picture.

The type of lesion generally found in cases of recurrent rheumatic fever we believe represents the course of a severe pericardial infection with acute exudative changes which, although not resulting fatally, were nevertheless too extensive to permit any complete restitution. In these cases such healing as occurred did not result in a completely normal structure but led to pericardial thickenings, adhesions and obliterations which in themselves were undoubtedly an unfavorable contributing influence on the course of the disease.

With this interpretation of the various pericardial pictures it would seem that almost in 100 per cent of the cases of rheumatic disease of the heart the pericardium was affected with certain inflammatory changes. However, only in a variable percentage of such

cases are these lesions severe enough to lead to the striking exudative membrane reactions that characterize the obvious pathological and clinical manifestations by which we recognize the acute rheumatic infection. For this reason, in chronic rheumatic cases, such as those found in Groups IV and V, there is no clinical history of rheumatic fever and little or none of the characteristic gross pathological appearances of that disease in the pericardium. Only careful microscopic examination reveals that even in these cases the pericardium has not been immune from rheumatic involvement.

### SUMMARY

There have been described in this report the gross and microscopic pericardial lesions found in 87 cases of active and inactive rheumatic fever. These cases were divided into five clinical groups depending on the course of the disease. It is shown that while each group may present individual differences, the lesions as a whole fall into three more or less characteristic histological patterns. Data on the incidence of the various lesions are presented in which it appears that rheumatic disease of the heart is almost invariably associated with certain inflammatory changes in the pericardium. A description is given of the age period changes in the histology of the normal pericardium.

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## DESCRIPTION OF PLATES

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### PLATE 21

FIG. 1. Structure of normal pericardium. Age  $6\frac{1}{2}$  years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = lamina propria. Note loose tissue separating this layer from overlying epithelial layer bound by delicate elastic membrane; B = adipose layer; C = myocardium.

FIG. 2. Superficial layers of visceral pericardium from active case of rheumatic fever. Age 19 years. Medium power. Hematoxylin and eosin stain.

A = proliferating epithelium; B = denuded area. Note beginning separation of epithelial layer from underlying tissue. C = loose tissue between epithelial layer and lamina propria showing edema and containing lymphocytes, polymorphonuclear leukocytes and congested capillaries; D = lamina propria showing swelling and eosinophilic change.

FIG. 3. Visceral pericardium from active case of rheumatic fever showing poly-poid and pseudogland formations. Age 39 years. Medium power. Hematoxylin and eosin stain. Note proliferating cuboidal epithelium and hemorrhage beneath the epithelial layer.



# PLATE 22

FIG. 4. Visceral pericardium from active case of rheumatic fever. Age 17 months. Low power. Hematoxylin and eosin stain.

A = lamina propria infiltrated with leukocytes and showing congested vessels in vicinity; B = moderately inflamed adipose layer; C = strip of myocardium; D = large coronary branch in adipose layer. Note inflammatory cell infiltration in lower zone of adipose layer.

FIG. 5. Superficial layers of visceral pericardium from active case of rheumatic fever. Age 13½ years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = organizing granulation tissue which has almost completely replaced exudate on surface of visceral pericardium; B = remains of unorganized fibrin; C = elastic lamella separating lamina propria from organizing exudate; D = considerably inflamed lamina propria showing marked infiltration and dilated blood vessels; E = adipose layer beneath the lamina propria showing thick fibrous septa between fat cells and lobules.

FIG. 6. Pericardial wedge between left auricular and ventricular myocardium. Age 20 years. Low power. Hematoxylin and eosin stain.

A = left auricle; B = crest of posterior wall of left ventricle; C = adipose tissue constituting the pericardial wedge; D = aggregation of inflammatory cells and fibrin; E = connective tissue replacement of exudate.

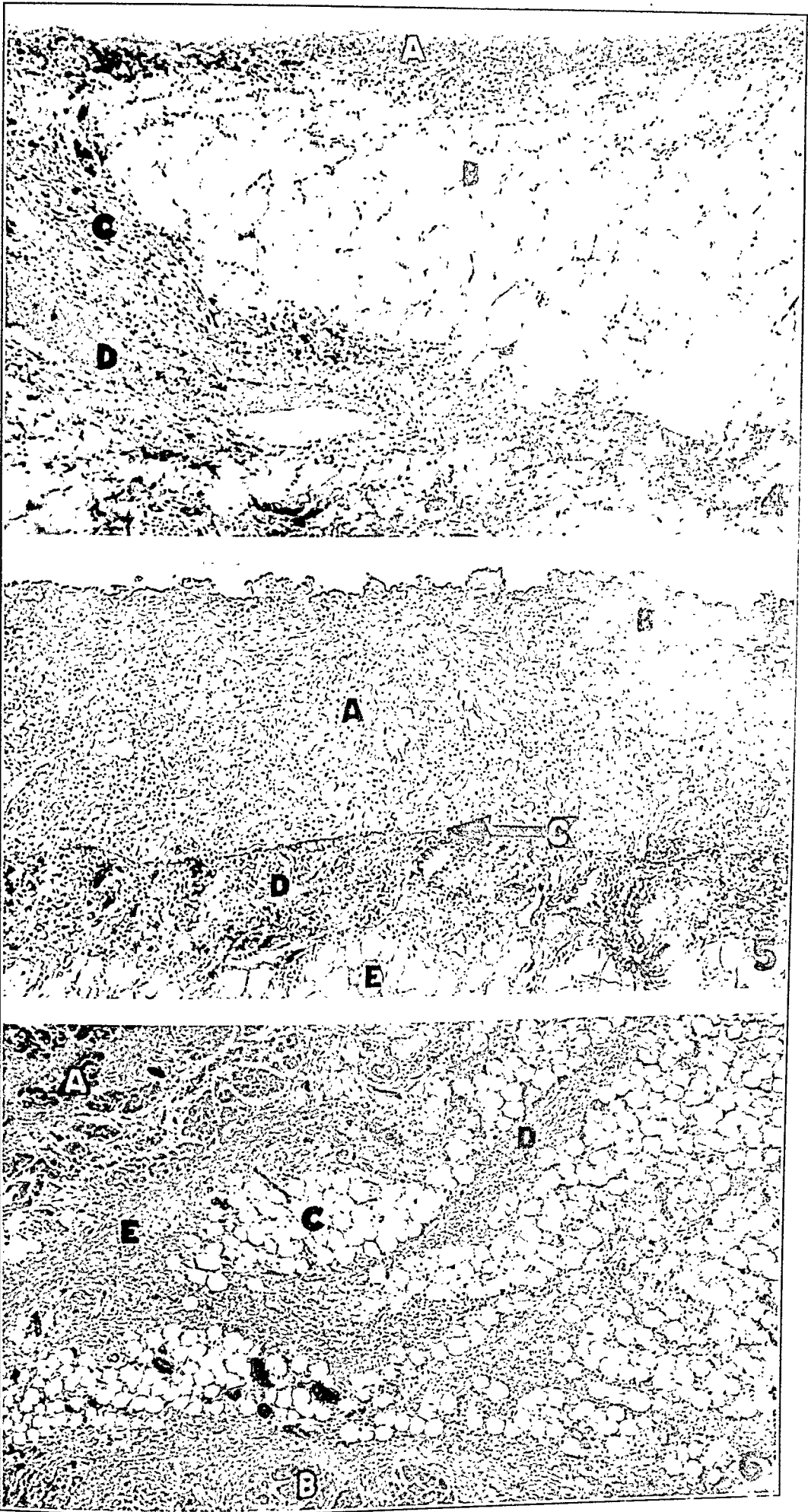




PLATE 23

FIG. 7. Visceral pericardium from active case of rheumatic fever. Age 20 years. Very low power. Weigert's elastic and Van Gieson's connective tissue stain.

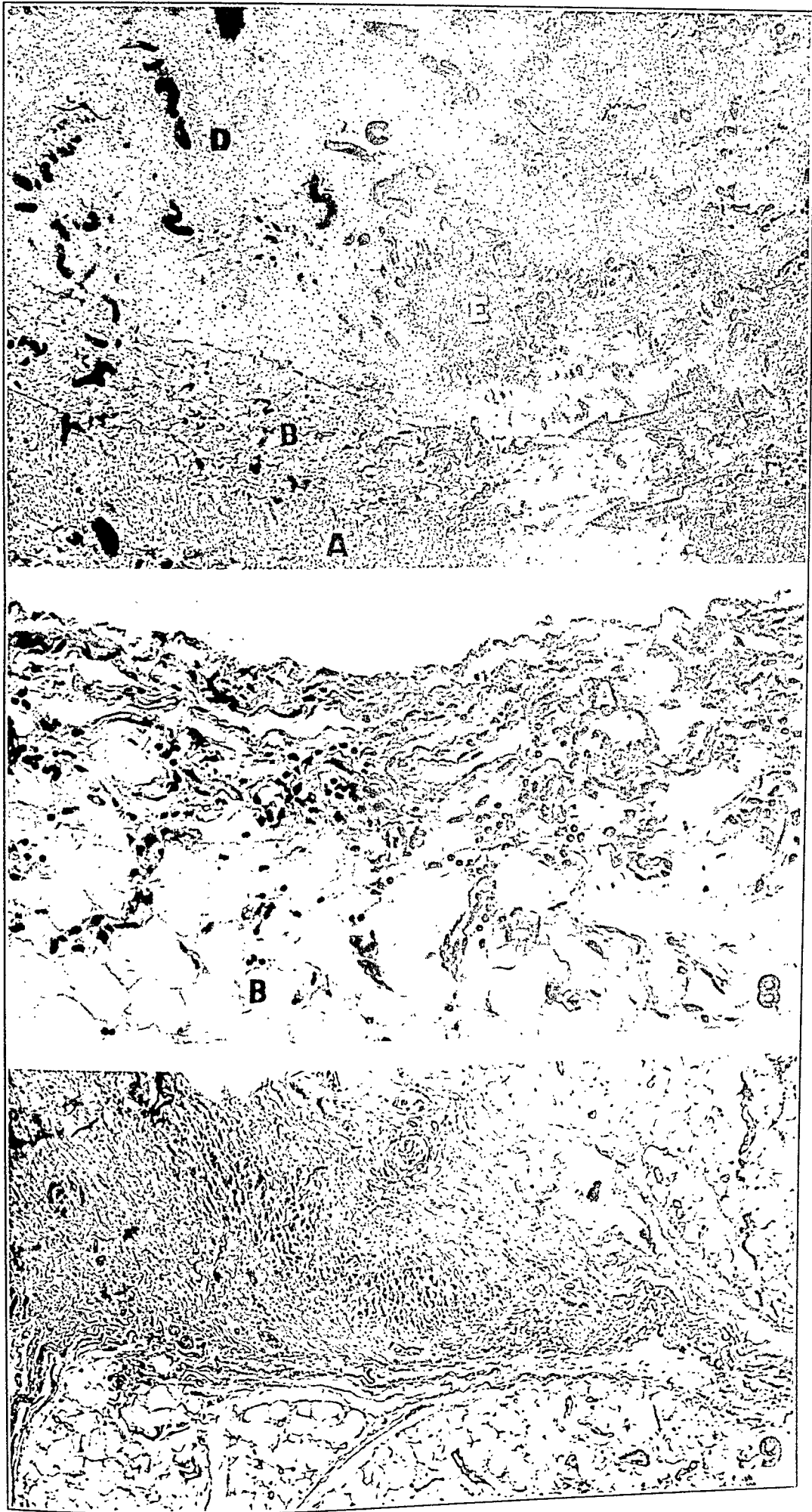
A = myocardium; B = visceral pericardium which has been almost entirely replaced by fibrous connective tissue. Note relatively few remaining fat cells, inflammatory cell infiltration and marked vascularization. Some of the blood vessels are seen to penetrate the upper limiting elastic lamella lying above the lamina propria (upper arrow). The lower arrow marks the level of the ventricular myocardial crest. C = edematous organizing and organized exudate superficial to the visceral pericardium; D = unorganized fibrin; E = hemorrhage.

FIG. 8. Superficial layers of visceral pericardium from inactive case of rheumatic fever. Age 42 years. Medium power. Hematoxylin and eosin stain.

A = lamina propria showing infiltration with lymphocytes. Note numerous engorged capillaries within the lamina propria and immediately below it; B = moderately inflamed adipose layer.

FIG. 9. Visceral pericardium from active case of rheumatic fever. Age 27 years. Low power. Hematoxylin and eosin stain.

Conglomerate Aschoff bodies (polarized type) embedded in adipose layer of visceral pericardium.





# SUBCLINICAL ADENOMA OF THE PITUITARY GLAND \*

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## INTRODUCTION

For many years the pituitary gland, or hypophysis cerebri, has been one of the favorite subjects for scientific investigation. In the early years of the present century interest in it, as indeed in most other organs, centered on its anatomical and pathological aspects. As physiological methods and knowledge improved, more and more attention was paid to its physiology, and in the past few years probably the chief interest has been in the latter field, particularly with reference to its effect on the other glands or organs of the endocrine system.

During this interval, however, and in spite of predominant interest in the physiology of the gland, several men, including Dandy and Rasmussen, investigating its anatomy, and Cushing and Bailey its pathology, have advanced our knowledge materially in these fields. The publication by Cushing<sup>1,2</sup> in 1932 of a series of cases describing a clinical syndrome often associated with basophilic adenoma re-awakened general interest in some of the pathological manifestations of the pituitary gland. This renewed interest centered particularly around those long neglected and minute adenomas which occur in the anterior part of the pituitary gland and which I propose to call sub-clinical adenomas. Up to the present these adenomas have been considered chiefly as pathological curiosities.

Adenomas of microscopic size or larger are found to occur rather frequently in the glands of the endocrine system. Adenomas in the thyroid gland are so frequent as to be almost normal findings. Adenomas of microscopic size or larger are found in at least 25 per cent of all suprarenal glands examined at autopsy. The kidney, while not an endocrine organ, may be the site of numerous tiny adenomas in

\* Abstract of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment for the degree of Master of Science in Neurology.

Received for publication October 21, 1935.

both the cortex and medulla and these adenomas may not produce any clinical symptoms. The prostate gland, again not an endocrine organ but one perhaps with some endocrine functions, shows a marked tendency to adenomatous formation with advancing age. For this reason it would be reasonable to suppose that symptomless adenomas in the pituitary gland should not be particularly uncommon.

It was not the purpose of this investigation to attempt to refute claims as to the clinical significance or insignificance of these adenomas. All of those in the present series were found in the pituitary glands of subjects who, at least so far as can be determined, had died of causes unrelated to disease in that gland. The study was undertaken chiefly to determine how frequently adenomas occur in supposedly normal pituitary glands, and incidentally to learn the ratio of frequency of adenomas composed of the three types of cells that are found normally in the anterior lobe, that is, chromophobic, eosinophilic and basophilic cells.

Such adenomas in the anterior lobe of the pituitary gland have been recognized for many years. Erdheim<sup>3</sup> in 1903 mentioned finding 2 adenomas of minute size in the hypophysis, both of which were apparently composed of basophilic cells. Löwenstein<sup>4</sup> in 1907 mentioned finding 2 more, but he neglected to mention their cell type. Erdheim and Stumme<sup>5</sup> in 1909, writing on the changes in the anterior lobe of the pituitary in pregnancy, reported finding 10 minute adenomas in 118 cases, or in 8.4 per cent. Roussy and Clunet<sup>6</sup> in their paper on tumors of the anterior lobe of the pituitary in 1911 also mentioned small adenomas found at autopsy that had apparently not caused clinical symptoms.

In spite of Erdheim and Stumme's<sup>5</sup> report of an incidence of approximately 10 per cent, these adenomas continued to be considered very rare until 1933, when Roussy and Oberling<sup>7</sup> stated that adenomatous foci occurred in 10 per cent of the pituitary glands examined at autopsy; however, they gave no statistics on the number of cases. In the same year Susman<sup>8</sup> found 23 adenomas in 22 of 260 pituitary glands from routine postmortem examinations, a percentage again of 8.4. Of these 23 adenomas 8 were composed of basophilic cells, 6 of eosinophilic cells and 5 of chromophobic cells; the remainder were not classified. These adenomas varied in size from 0.2 to 25 mm. In 1934 Close<sup>9</sup> reported that the incidence of

pituitary adenomas was approximately 10 per cent in a series of routine postmortem examinations, but that it ran as high as 44 per cent in those cases in which there was associated carcinoma in other glands. Many others, such as Stolkind,<sup>10</sup> Wall and Hoyle,<sup>11</sup> Pritchard,<sup>12</sup> Rutishauser,<sup>13</sup> Weber,<sup>14</sup> Russell, Evans and Crooke,<sup>15</sup> Moehlig,<sup>16</sup> Bishop and Close,<sup>17</sup> Teel,<sup>18</sup> Wieth-Pedersen,<sup>19</sup> Kraus,<sup>20</sup> Reichmann,<sup>21</sup> Anderson,<sup>22</sup> Bauer and Wassing,<sup>23</sup> Roch,<sup>24</sup> Craig and Cran,<sup>25</sup> and Ulrich,<sup>26</sup> have reported 1 or more cases of basophilic adenoma associated with the syndrome known as pituitary basophilism.

### SOURCE AND PREPARATION OF MATERIAL

The material for this study consisted of 1000 pituitary glands obtained over a period of years in the course of routine postmortem examinations in which permission to examine the brain had been granted. The pituitary gland was removed in these cases by clipping off the posterior clinoid processes with bone forceps and removing the gland *in toto*. It was then placed in 10 per cent formalin solution and stored.

In preparing the sections the glands were removed from the formalin and cut into sagittal sections paralleling the longest axis. Since the glands vary greatly in shape and size, this method resulted in sections from some glands being parallel to the anteroposterior diameter, whereas others were transverse. The sections were cut by hand in thicknesses varying from 1 to 1.5 mm. Since the majority of adenomas were found to be 1.5 mm. or larger in diameter, it was felt that by this method the likelihood of many being missed on section was fairly remote. It might be mentioned at this point that attempts to identify adenomas in the freshly cut sections were in most cases futile.

These sections were then placed in Zenker's fluid for 24 hours to facilitate later staining by the Mallory-Heidenhain method. They were then embedded in paraffin in the ordinary manner and two sections, 10 microns in thickness, were cut from each paraffin block and these were mounted and stained with hematoxylin and eosin in the usual way. By this method three to ten sections from each gland were stained. The hematoxylin and eosin sections were examined grossly and microscopically, and in those cases in which adenomas were found another section was cut from the corresponding block of tissue

and stained by the Mallory-Heidenhain method, as outlined by Kernohan,<sup>27</sup> to determine the types of cells composing the adenoma. With this stain the cytoplasm of the chromophobic cells either does not stain at all or is pale blue; the eosinophilic granules stain bright red and the basophilic granules stain dark blue. These sections were then used to classify the adenomas as to their cell types.

### CHARACTERISTICS OF ADENOMAS

At this point it becomes necessary to consider what constitutes an adenoma. The basophilic cells in particular have a tendency to occur normally in clumps or islands, which on superficial examination look like small adenomas but are probably merely normal architectural variations. Probably there can be no absolute criterion by which one can differentiate true adenoma formation and unusual structural variations. The smaller adenomas in the anterior lobe of the pituitary have no demonstrable capsule, and even in the larger ones what at first glance appears to be a capsule is merely a condensation of the cells surrounding the adenoma. In the majority of cases, however, there is no difficulty in distinguishing between an adenoma and a structural abnormality. The majority of adenomas found were spherical or nearly so (Fig. 1) and the larger ones were well demarcated by the ring of compressed pituitary cells surrounding them.

Another distinguishing factor is that in an adenoma the percentage of one particular type of cell in a given area is a great deal higher than in the normal portions of the gland. Moreover, the cell pattern differs from the normal arrangement of acini bordering vascular sinuses. In some of the adenomas the pattern resembles the honeycomb arrangement of pavement epithelium (Fig. 2); in others it looks like a markedly convoluted papilloma (Fig. 3) or compound tubular gland (Fig. 4). In still others there is no apparent structure, but the cells appear to be scattered loosely around a few vascular sinuses, with little connective tissue framework. Some also reveal areas of degeneration, necrosis and vacuole formation.

Just as the structure of the adenomas varies greatly, so do the cells show marked variability in appearance in different adenomas. In some, as in those appearing to be composed of pavement epithelium (Fig. 2), the cells are large and clear with centrally placed

and somewhat pale staining nuclei. In others the cytoplasm is somewhat scanty, the outline of the cell irregular, and the nucleus eccentrically placed. While the cells differ greatly in appearance in different adenomas, even in those made up of the same type of cells, in general they are fairly constant in a given adenoma. It was also noted that there was marked variability in the staining characteristics of different adenomas of the same type. In the eosinophilic and basophilic adenomas the color ranged from pale pink to deep red in the former and from pale lavender to deep blue in the latter. The cell types were determined by the presence of typical granules. Variations in color were not due to faulty staining technic, for the normal parts of the gland which were used as controls stained normally.

It was not found that the adenomas had any definite site of predilection for the anterior lobe, although the majority tended to be more peripherally than centrally located. Some projected from the surface of the gland under the capsule (Fig. 5), whereas others were embedded deep in the substance of the gland. Many occurred along the border contiguous to the pars intermedia, and in this region they tended to lose their spherical shape and become flattened at the intermedial pole (Fig. 6).

Adenomas were found composed of each of the three types of cell normally found in the pituitary gland. Rasmussen<sup>28-30</sup> has found that, in a series of pituitary glands from adult males, the anterior lobe contains on the average of 52 per cent chromophobic cells, 37 per cent eosinophilic cells and 11 per cent basophilic cells. The proportion in the glands of adult females is only slightly different from that in males. One would therefore expect that if these three types of cell were all actively growing and reproducing themselves, as some histologists believe, then in a large series of cases adenomas composed of the different types of cells should occur in about the same ratio of frequency as the cells themselves normally occur. This, however, was not found to be the case, as will be shown later.

Experience with operative specimens of pituitary tumors has shown that they are frequently composed of mixed cells. This is also true of subclinical adenomas. Not more than two or three adenomas in the series were composed of purely one type of cell; the majority, on the other hand, had one type predominating sufficiently to classify the adenoma according to this predominant type of cell. In a few



cases, however, two or even three types of cells occurred in almost equal proportions in the same adenoma, so it was necessary to classify them merely as mixed types.

In some cases more than 1 adenoma was found in a single gland. In several of these cases 2 adenomas were found to be associated, in some cases being similar types; in other cases two different types of adenoma were present. In 2 cases, 3 distinct adenomas were found, and in 1 of these cases all three types of adenoma were represented. In another case several adenomas were found, of various sizes and representing various types of cells; semiserial sections indicated that there were 10 or more separate and distinct adenomas in this one pituitary gland.

### FREQUENCY OF OCCURRENCE

Of the 1000 pituitary glands examined, 225 were found to contain 1 or more adenomas. In 224 of these glands, excluding the one with the multiple adenomas, there were found to be 265 adenomas. These adenomas were classified as follows: chromophobic, 140 (52.8 per cent); eosinophilic, 20 (7.5 per cent); basophilic, 72 (27.2 per cent); and mixed types, 33 (12.4 per cent).

### AGE INCIDENCE

In an endeavor to find the age group in which adenomas most frequently occurred, the ages of those subjects with adenomas were plotted against the age curve for the 1000 subjects in the whole series. These latter had ranged from stillborn infants to a man of 99 years. The youngest subject in whom adenomas were found was 2 years old, the oldest was 86 years old.

As can readily be seen from Chart 1, the greatest incidence of adenomas occurred in the sixth decade of life; however, since the greatest amount of autopsy material also occurs in this decade, the curve in Chart 2 was plotted from the ratio of adenomas to pituitary glands in each age group. This shows even more conclusively that the highest incidence of such adenomas occurs in the sixth decade of life (Chart 2). Because of the relatively small number of subjects who were more than 70 years of age, the end of the curve is indefinite and inconclusive.

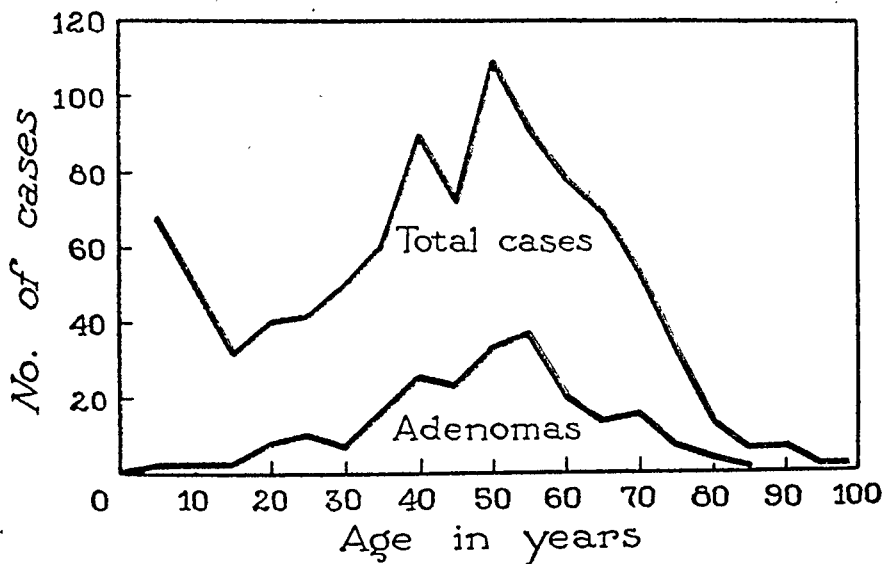


CHART 1. The ages in which adenomas occurred and the age groups in which the pituitary glands were obtained.

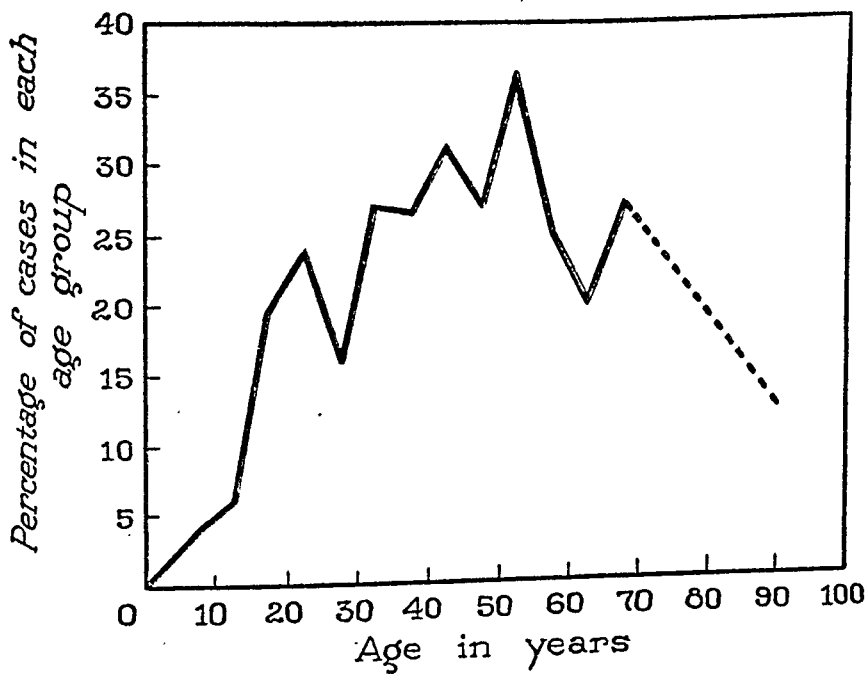


CHART 2. This graph was plotted by dividing the number of adenomas found in each age group by the total number of pituitary glands derived from subjects in that age group.

### SEX INCIDENCE

Of the 225 cases in which adenomas were found, 148, or 66 per cent, of the patients were males, 77, or 34 per cent, females. This would seem to indicate the predominance of adenomas among males. However, since the proportion of males to females in the 1000 cases was 63 to 37 per cent, it can be seen that the incidence in the two sexes is about equal.

### CLINICAL FEATURES

As has been mentioned before, the 1000 pituitary glands were obtained in a series of routine postmortem examinations in which permission to examine the cranial cavity had been granted. The clinical diagnoses and causes of death in these cases ranged through almost the entire list of medical and surgical conditions. As one would suspect from the age curve, diseases of middle life, such as cardiac failure, hypertension, nephritis and carcinomatosis, were more frequent than some other conditions, although the causes of death ranged all the way from stillbirth, through suicide, to senility. In the 225 cases in which adenomas were found the same variability in clinical findings and causes of death were apparent. The most notable thing about them was that there was nothing in the history or clinical findings in any case in which an adenoma was found to suggest the presence of any pituitary dysfunction. This was true in cases in which basophilic or eosinophilic adenomas were present as well as in those in which the adenomas were chromophobic. This is somewhat startling since in a few cases the adenomas were so large as almost to destroy the gland. Apparently, however, there was sufficient normal tissue remaining to sustain normal pituitary function.

### PROPORTION OF DIFFERENT TYPES OF ADENOMA

As can be seen from the percentages of the different types of adenoma, the chromophobic adenomas occurred in about the same proportion as the chromophobic cells themselves occur in normal glands. With the eosinophilic and basophilic adenomas, however, there is considerable discrepancy. Although eosinophilic cells constitute 37 per cent of the normal hypophyseal cells, the eosinophilic adenomas represent only 7.5 per cent of the total number of adenomas. On the other hand, although the basophilic cells comprise only

11 per cent of the average normal cell content of the anterior lobe, basophilic adenomas made up 27 per cent of the total group of adenomas. It might be imagined that if the eosinophilic adenomas are more active than the other types, they might grow faster and give clinical symptoms early, since the majority of clinically recognized chromophilic adenomas occur before the age of 50 years. However, in the group of adenomas reported here the average age was greater than 50 years, and so this explanation is not warranted.

Of the 33 mixed types of adenomas, 21 were composed of mixed basophilic and eosinophilic cells; the remaining 12 consisted of chromophobic and basophilic cells with very few eosinophils.

#### SUMMARY AND CONCLUSIONS

It has been shown, therefore, that small adenomas of the anterior lobe of the pituitary gland, instead of being relatively rare pathological curiosities, occur with considerable frequency, since they occurred in 22.5 per cent of a series of 1000 unselected cases, or in nearly 1 out of every 4. Thus, the anterior hypophysis is shown to act much like other glands of the endocrine system in its tendency toward formation of adenomas.

It has been shown further that, like adenomas in other organs of the body, adenomas of the anterior lobe of the pituitary gland occur with increasing frequency in the higher age groups, and that they also apparently occur in the same proportion in both sexes.

It has also been shown that the majority of adenomas of the anterior lobe of the pituitary, irrespective of type, are entirely benign and give no recognizable clinical symptoms, and that they can be demonstrated only by rather detailed examination of the gland at autopsy. This would justify the term "subclinical adenoma" as applied to them. It is entirely possible, however, that under the influence of some unknown stimulus some of these adenomas may secrete a hormone or similar substance capable of producing clinical symptoms.

The chromophobic adenomas have been seen to occur in about the same relative proportion in the series as do the chromophobic cells themselves in the average normal gland. However, the eosinophilic and basophilic adenomas do not occur in the same relative proportions as their respective cells do in the average normal gland, and an adequate explanation for this has not been found.

Future work is yet to be done on the group of basophilic adenomas in this series in an attempt to tabulate and correlate their clinical features.

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## DESCRIPTION OF PLATE

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### PLATE 24

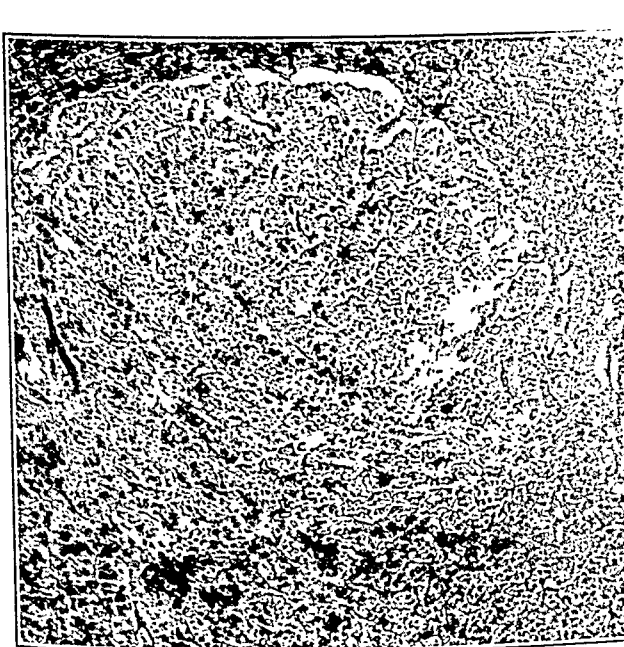
- FIG. 1. Chromophobe adenoma. The tendency to spherical shape, as well as the condensation of cells around the periphery, forming a pseudocapsule, is evident. Hematoxylin and eosin stain.  $\times 23$ .
- FIG. 2. Chromophobe adenoma. The adenoma cells arranged like pavement epithelium. Mallory-Heidenhain stain.  $\times 300$ .
- FIG. 3. Basophilic adenoma. The arrangement of cells resembles that of a convoluted papilloma. Mallory-Heidenhain stain.  $\times 48$ .
- FIG. 4. Chromophobe adenoma. The resemblance to a compound tubular gland is evident. Mallory-Heidenhain stain.  $\times 85$ .
- FIG. 5. Chromophobe adenoma. The adenoma budges the capsule of the pituitary gland. Hematoxylin and eosin stain.  $\times 18$ .
- FIG. 6. Eosinophilic adenoma. The adenoma is triangular and one side is against the pars intermedia of the pituitary gland. Hematoxylin and eosin stain.  $\times 60$ .



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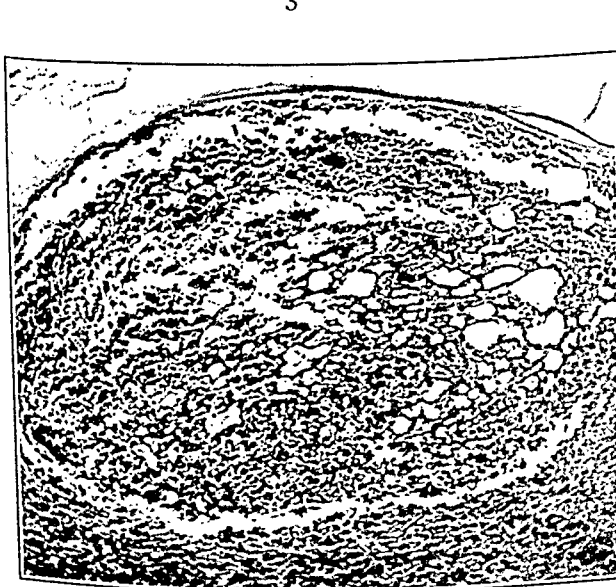
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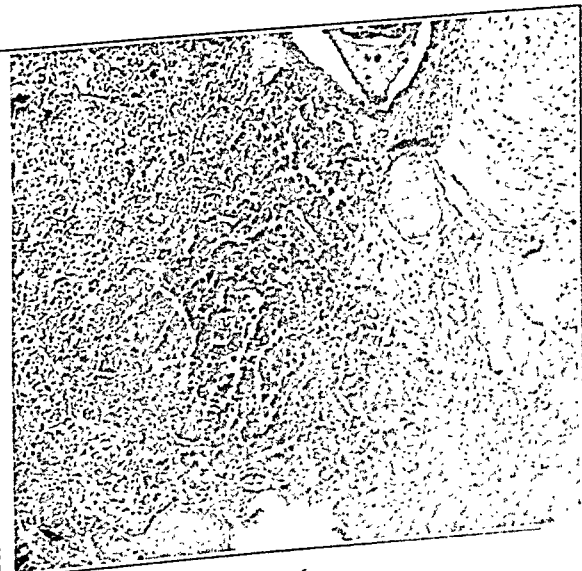
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# NUCLEAR ALTERATIONS FOLLOWING INTRAVENOUS INJECTIONS OF GLUCOSE AND OF OTHER SOLUTIONS \*

JACK LEE

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In the hope of learning more about intranuclear inclusions in virus diseases, workers in this laboratory have proceeded along several different lines. Employing the Feulgen reaction and the Macallum test for masked iron, it has been found that the inclusions contain little or no thymonucleic acid and iron.<sup>1</sup> By methods of microincineration it has been shown that they consist of material that can be completely burnt away without leaving a noticeable mineral residue.<sup>†2,3</sup> With the aid of the ultracentrifuge<sup>4</sup> the specific gravity of the inclusion material has been found to be less than that of basophilic chromatin and clear nuclear sap. Inclusion-like bodies have been reported in various experiments in which viruses are apparently not involved.<sup>5, 6</sup>

The purpose of the experiments herein recorded is to determine how closely experimental inclusions can be made to resemble those caused by viruses.

The literature should be considered briefly. Some investigators, in reporting experiments not primarily concerned with virus diseases, have described nuclear alterations so like those caused by viruses as to call for comment; while others have made experiments especially designed to lead to nuclear modifications which would be indistinguishable from nuclear inclusions in virus diseases.

In 1926 Luger and Lauda<sup>7</sup> described and illustrated nuclear inclusions in salvarsan dermatitis which resembled very closely the type of inclusions in herpes. Cole and Kuttner,<sup>8</sup> however, took the view that before these could be accepted as an expression of salvarsan dermatitis, it would be necessary to demonstrate the absence of a filterable virus. In other words, they believed that when typical intranuclear inclusions are found, the presence of a virus is to be assumed unless its absence is proved experimentally. Cowdry,<sup>9</sup> on the other hand, has stated that the presence of intranuclear inclusions should not be taken

\* Aided by an appropriation from a grant by the Rockefeller Foundation to Washington University for research in science and a grant from the Rockefeller Foundation for research in virus diseases.

† A slight residue was observed from salivary gland inclusions by Scott, Gordon H. Sur la localisation des constituants minéraux dans les noyaux cellulaires des acini et des conduits excréteurs des glandes salivaires. *Compt. rend. Acad. d. sc.*, 1930, 190, 1073.

Received for publication October 3, 1935.

at face value as indicating the action of some filterable virus. He<sup>10</sup> also entertained the possibility that the inclusions may eventually be produced artificially without viruses. As nuclear inclusions form, the acidophilic material giving rise to them accumulates in the center of the nucleus and the basophilic chromatin migrates to the periphery and marginates on the nuclear membrane. Cowdry<sup>10</sup> has suggested that this process resembles to some extent chromatolysis in nerve cells and "chromatolysis by margination" in ovarian granulosa cells. Thinking that an enzyme might be involved he attempted without success to produce nuclear inclusions by the injection of nuclease prepared by Dr. D. J. Kooyman (personal communication).

Cowdry and Kitchen,<sup>11</sup> in testing the specificity of the nuclear inclusions in vesicular stomatitis, injected trypsin, pepsin, hydrochloric acid and various other fluids intradermally into the footpads of guinea pigs, but no artificial nuclear inclusions closely resembling the type inclusions of herpes were produced.

Of considerable significance are certain experiments on nerve cells. In 1929 Akiyama,<sup>12</sup> in a paper of which I have seen only reviews, reported a shrinkage of nucleoplasm away from the nuclear membrane, so that the central mass of material became surrounded by a clear halo something like that which often envelops a typical nuclear inclusion. He observed this modification as a result of subjecting nerve cells to galvanic stimulation plus ammonium ion. The following year Heinbecker and O'Leary<sup>5</sup> described, independently of Akiyama, at a meeting of the American Association of Anatomists, comparable nuclear alterations in several types of nerve cells after electrical excitation of their processes. They remarked upon the resemblance between the centrally placed chromatin masses and nuclear inclusions in virus diseases. In 1931 Davenport, Ranson and Terwilliger<sup>13</sup> discussed the observations of Akiyama, and of Heinbecker and O'Leary, and presented the results of their own experiments on the influence of drying and of hypertonic salt solutions on nerve cells. By these means a similar central clumping of nuclear material was produced which they illustrated. They suggested that "... the nuclear inclusions observed pathologically may be the result of disturbed osmotic conditions in the cell." Lee,<sup>6</sup> then, injected cats intravenously with a number of solutions calculated to alter osmotic relations and discovered various nuclear modifications but did not claim "... that any of the changes are identical with those caused by viruses though they resemble them in certain particulars."

More recently Cowdry and Scott,<sup>14</sup> while investigating renal lesions in monkeys given activated ergosterol 10,000 x, observed that nuclear inclusions were more frequently met with in the tubular cells of treated than of control animals. Since they occurred in 12 out of 16 treated, as contrasted with 1 out of 10 normal controls and 18 out of 107 pathological controls consisting of monkeys employed in the laboratory in a variety of experiments but not given activated ergosterol, the authors concluded that treatment "may have activated or intensified a process already latent in the kidneys. . . ."

In addition to chance observation of inclusions in the course of experiments and to purposeful attempts to produce them in the absence of virus, it is necessary to mention reports of their presence in tissues not subjected to any unusual procedure whatsoever. It is conceivable that the nuclei in normally functioning cells, which must adjust themselves to their particular environments (osmotic and otherwise), may on regular occasions be modified in such a way that they look as if they had been subjected to virus action. Confirmed observation of

this phenomenon would have a bearing on the principal problem of the significance of nuclear inclusions in virus diseases almost as direct as their experimental formation. Acidophilic intranuclear bodies, reminiscent of those in Borna disease and poliomyelitis, but not much like those in herpes — to which it is always necessary to refer as if to a standard — have indeed been repeatedly recorded in the epididymis<sup>15-18</sup> and in the nucleus supra-opticus and paraventricularis of the midbrain.<sup>19, 20</sup>

Obviously, however, the value of each and all of the above mentioned findings depends upon the precise degree of similarity between the structures noted and nuclear inclusions proved to be brought about by viruses.

My thanks are due Doctors E. V. Cowdry and James L. O'Leary for encouragement and advice.

#### NORMAL STRUCTURE OF THE NUCLEI SELECTED FOR EXPERIMENT

Although nerve cells of various sorts were examined, Purkinje cells of the cerebellum of cats were chosen as the principal material because they are conveniently disposed in a single layer, are of fairly uniform size and shape, and possess large nuclei.

After ordinary fixatives, such as the fluids of Zenker, Carnoy, Bouin and Regaud, and coloration with hematoxylin and eosin or by Giemsa's stain, by far the most conspicuous nuclear component is the nucleolus (Figs. 1-3). Usually there is only one per nucleus and it is called an amphinucleolus for the reason that it is colored by both acid and basic dyes. The central core, which is more or less spherical, is acidophilic and on this is plastered in an irregular way a small amount of amphophilic material. In the ground substance between this amphinucleolus and the nuclear membrane a few tiny particles of acidophilic and basophilic material are generally visible. As a rule, the nuclear membrane colors only with "acid" dyes. The Purkinje cell nuclei resemble those of other large nerve cells in the spinal ganglia, anterior horns and other locations in being much less basophilic than most nuclei of corresponding size of non-nervous tissues.

Very little is known of the actual structure of the Purkinje cell nuclei *in vivo*. Examination of living cells by the method of dark-field illumination reveals no particles of nuclear material between the nucleolus and the nuclear membrane. The nucleolus, as in fixed material, is the most conspicuous nuclear component and the only one the existence of which has been demonstrated in living inter-

kinetic nuclei by the method of microdissection.<sup>21</sup> Bensley,<sup>22</sup> however, upon examining the nuclei of several different kinds of cells, after preparation by Gersh's<sup>23</sup> modification of the Altmann freezing and drying technique, concludes that "nuclei which are optically homogeneous in the living state are not in fact homogeneous but exhibit a chemical structure due primarily to the segregation of the chromatin in special locations in the interior of the nucleus." Preparations of the cerebellum made by this new technique showed particulate material in the nuclear ground substance, as Bensley has reported, and of which no indication is visible when viewed by direct or oblique illumination. At present each investigator must decide for himself which method is likely to reveal the true condition of affairs in the nuclei of living nerve cells — that of Altmann or of direct study of unfixed, still living tissue.

Care was taken to establish the range of variation in nuclear structure in a single individual and in different individuals, which could be regarded as the principal control. Tissues were excised from as nearly the same part of the vermis as possible and the Purkinje cells from the crest of each folium were chosen for study. Most of the variation in a single individual was in the arrangement of both acid and basic staining chromatin granules. In some nuclei these were distributed in an even fashion in the area between the nucleolus and the nuclear membrane; while in others most of them were concentrated in the zone around the nucleolus. Fine strands of acid staining material often stretched between these and a few particles just within the nuclear membrane. This left the intermediate zone relatively clear.

Sections of the cerebellum from 103 cats were examined. Twenty-five were normal. The others had been used in the laboratory for experimental purposes. Included in them were some that had undergone different degrees of vascular occlusion, for which I am grateful to Doctor Louis D. Tureen; others had received injections of various solutions directly into the pancreas or liver. In the Purkinje cells from this group of 103 animals there was a variation in both the amount and distribution of intranuclear chromatin, as well as the size and shape of the nuclei. Some nuclei were small and contained small clumps of both acid and basic staining substance, while others were larger and possessed scarcely any basophilic particles and a minimum of acid staining ones. In sections from one animal

the smaller nuclei would predominate while in another both large and small were present in about the same percentage. In all of the tissue examined approximately 65 per cent of the nuclei were of the small variety. In both types of nuclei, of these control sections, fine strands connected the acid and basic staining granules with the nuclear membrane, leaving no clear area between the two.

#### INTRANUCLEAR AGGREGATES PRODUCED EXPERIMENTALLY

The experiments are listed in Table I. The injection of the various solutions was made with a syringe or burette connected directly with the femoral vein. The hypertonic solutions consisted of glucose, glucose plus acacia, sodium chloride and bicarbonate. Distilled water was injected as the hypotonic solution. All cats used in these experiments, including the controls, were killed by ether and exsanguination with the exception of those that died during the process of injection.

Small pieces of tissue were removed and fixed with 10 per cent formalin, Zenker's fluid containing 5 per cent acetic acid, 10 per cent formalin, and a solution containing 9 parts of absolute alcohol and 1 part formalin. They were then dehydrated, cleared, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin or Giemsa.

#### (A) *Glucose*

Histological examination of tissues from Cats 1 and 2, which were killed immediately following the intravenous injection of glucose, showed definite nuclear alterations in cells of the spinal and sympathetic ganglia and spinal cord, Purkinje cells of the cerebellum, and in pyramidal cells throughout the thickness of the cerebral cortex. No comparable alterations were seen in sections of pancreas, spleen, liver, adrenal, lymph node, lung, thyroid, parotid and submaxillary gland from 6 animals that had received glucose intravenously. Tissues of the others, given glucose, were not examined.

In the Purkinje cells the nuclear material formed a fairly compact mass about the nucleolus as indicated in Figure 4. The mass was separated from the nuclear membrane by a large clear zone, strongly suggestive of the "halo" about the intranuclear inclusions in virus diseases. Intermediate stages with threads reaching from

TABLE I  
*Summary of Experiments*

Cat. No.	Solution injected	Period of injection	Time of death after injection
1	120 cc. 50 per cent glucose	1 hour, 15 minutes	Immediately
2	130 cc. 50 per cent glucose	1 hour, 30 minutes	Immediately
3	120 cc. 50 per cent glucose	1 hour	1 hour
4	130 cc. 50 per cent glucose	1 hour	1 hour
5	130 cc. 50 per cent glucose	1 hour, 15 minutes	2 hours
6	120 cc. 50 per cent glucose	1 hour	2 hours
7	120 cc. 50 per cent glucose	1 hour	3 hours
8	120 cc. 50 per cent glucose	1 hour	3 hours
9	130 cc. 50 per cent glucose	1 hour, 20 minutes	4 hours
10	120 cc. 50 per cent glucose	1 hour	4 hours
11	120 cc. 50 per cent glucose	1 hour	24 hours
12	130 cc. 50 per cent glucose	1 hour, 30 minutes	3 days
13	120 cc. 50 per cent glucose	1 hour	5 days
14	120 cc. 50 per cent glucose plus 12 per cent acacia	2 hours	Immediately
15	120 cc. 50 per cent glucose plus 12 per cent acacia	2 hours, 30 minutes	Immediately
16, 17	150 cc. 30 per cent sodium chloride	1 hour, 30 minutes	Immediately
18, 19, 20, 21	50 cc. saturated sodium bicar- bonate in distilled water	Animals died dur- ing injection	
22, 23, 24	300 to 400 cc. distilled water	2 hours	Injection continued until death

the shrunken chromatin mass to the nuclear membrane were seldom seen. The nucleoli were smaller than those of normal controls, stained heavily with hematoxylin and often could not be differentiated in staining qualities from the shrunken mass of nuclear material by which they were surrounded.

In Cats 3 and 4, which were killed 1 hour after the injection of glucose, nuclear modifications similar to those seen in Cats 1 and 2 were noted. As represented in Figure 5, the chromatin granules in the nuclei of the Purkinje cells formed a dense mass about the nucleolus. Both cells and nuclei were slightly larger than those of normal controls (Table II). The nucleoli were likewise swollen and vacuolated. Sometimes they could not be distinguished because they were completely masked by the nuclear material about them. The measurements were made in the following manner:

Fifty Purkinje cells from the cerebellum of a normal cat and of cats killed immediately and 1, 2, 3 and 4 hours after the injection of glucose were measured by the method of Scammon and Scott.<sup>24</sup> With the aid of a camera lucida the cells were outlined with India ink on "Kodaloid, No. 3" (Eastman). The outlines were cut out, placed in a desiccator for 24 hours and then weighed. The first row of figures in Table II gives the average weight of the cellular outlines in grams. These show that there is an initial decrease in cell volume immediately after injection, followed by an increase which reaches a maximum at 2 hours, and by 4 hours after injection is again within the range of normal variation in the material.

The greatest diameter of 100 Purkinje cell nuclei from the same 5 cats was measured in microns by an ocular micrometer. The second row of figures in Table II gives the averages. They show that there is an initial decrease in the size of the nuclei, followed by a gradual rise which reaches the maximum 2 hours following injection. By 3 hours after injection there is a marked decrease followed by a rise to normal within 4 hours.

The most pronounced swelling of both cells and nuclei was seen in tissues from Cats 5 and 6 which were killed 2 hours after the administration of glucose (Table II). In these cells (Fig. 6) the nucleoplasm formed an irregular granular mass about the nucleolus as in those animals killed earlier than 2 hours after injection. The intranuclear mass remained separated from the nuclear membrane by a "halo." The nucleoli, when they could be distinguished, were enlarged, vacuolated, and did not stain as heavily with hematoxylin and eosin as those of animals killed 1 hour after injection.

In sections from Cats 7 and 8, which were killed 3 hours after injection, the nuclei were much smaller than in the 2 hour animals and



slightly smaller than those of normal controls (Table II). It was apparent (Fig. 7) that the nuclear wall had partially collapsed, allowing the nuclear material to fill the entire nucleus, thus obliterating the "halo." The nuclear chromatin was grouped in large clumps throughout the nucleus and strands of nuclear material adhered to the nuclear membrane. The cells had likewise shrunk, leaving a large, clear, pericellular space. The nucleoli remained slightly enlarged and were plastered with chromatin particles.

Examination of the nuclei of Purkinje cells from Cats 9 and 10, which were killed 4 hours after injection (Figs. 8, 9 and 10), and a

TABLE II  
*Variation in Size of Cells and Nuclei*

Condition of animal	Average weight of cellular outlines	Average nuclear diameter
Normal .....	gm. 0.6147	$\mu$ 11.3560
Killed immediately after injection .....	0.5563	10.9384
Killed 1 hour after injection .....	0.7698	11.9455
Killed 2 hours after injection .....	0.9287	12.4375
Killed 3 hours after injection .....	0.7161	11.2260
Killed 4 hours after injection .....	0.6427	11.5380

comparison of these with normal controls showed practically the same distribution of chromatin and size of the nuclei as in normal controls.

In Cats 11, 12 and 13, which were killed at 1, 3 and 5 day intervals after glucose injection, the deposition of nuclear chromatin showed no more deviation from the mean than did the normal control material from different individuals.

Five micron sections of the material, which had been fixed in 90 per cent alcohol containing 10 per cent formalin, were incinerated by the method of Policard,<sup>25</sup> as modified by Dr. Gordon H. Scott,<sup>26</sup> to whom I am much indebted for aid in this connection. The normal ash content of the nerve cell nucleus was compared with that of animals killed immediately and 1, 2, 3 and 4 hours after the injection of glucose. The masses of nuclear substance showed a high ash content in contrast with known virus inclusions which were incinerated in

this laboratory by the same method (Cowdry,<sup>2</sup> and L. E. and E. J. Rector<sup>3</sup>).

Thymonucleic acid, as identified by the Feulgen reaction (Cowdry<sup>1</sup>), was confined to the intranuclear mass. It was most apparent about the nucleolus in animals killed immediately, and gradually dispersed in those killed 1, 2, 3 and 4 hours after injection. Bensley<sup>22</sup> has shown that thymonucleic acid is evenly distributed in normal cells prepared by the Altmann-Gersh procedure.

### (B) *Glucose Plus Acacia*

It is known that acacia, when injected with a glucose solution, tends to hold for some time the fluid that is drawn into the blood stream. To determine whether or not the mixture would produce the same effect on nuclei as a glucose solution, 2 cats were injected with 120 cc. of 50 per cent glucose and 12 per cent acacia. In sections from these cats (14 and 15) nuclear alterations, like those of glucose-injected animals, occurred in the cells of spinal ganglia, anterior horns of the spinal cord, Purkinje cells of the cerebellum and in pyramidal cells throughout the thickness of the cerebral cortex. No sympathetic ganglia were examined. In most of the altered nuclei there was less shrinkage of the nuclear material and more strands of chromatin connecting the shrunken nuclear mass with the nuclear membrane than in glucose-injected animals.

### (C) *Sodium Chloride*

Tissues from Cats 16 and 17, which were killed immediately following the injection of sodium chloride, exhibited nuclear changes similar to glucose and glucose plus acacia-injected animals, except that the nuclear chromatin was more acidophilic and less concentrated about the nucleolus than in the animals killed immediately after glucose injection.

### (D) *Sodium Bicarbonate*

Fifty cc. of a saturated solution of sodium bicarbonate in distilled water were injected into each of Cats 18, 19, 20 and 21. All died during the process of injection. Microscopic examination revealed nuclear alterations in the nervous system comparable to those seen in the glucose-injected animals. No changes were noted in other or-

gans examined, such as liver, pancreas, spleen, adrenal, kidney and submaxillary gland. The cells of Purkinje were the only cells of the cerebellum affected. Their shrunken nuclear chromatin was finely granular and distinctly more acidophilic than that of the glucose treated animals. In the slightly swollen nuclei of cells from the anterior horns, spinal and sympathetic ganglia, the nuclear substance also formed a shrunken granular mass about the nucleolus in which were deposited from one to four acidophilic staining droplets, slightly smaller than the nucleoli, with irregular edges. The nucleoli remained apparently unmodified.

### *(E) Distilled Water*

Examination of tissues from Cats 22, 23 and 24, which were injected with between 300 and 400 cc. of distilled water until death, brought to light nuclear changes in the cells of the spinal ganglia and anterior horns of the spinal cord. No alterations were apparent in any of the cells of the cerebellum, cerebral cortex, liver, pancreas, spleen, testis, ovary, kidney, adrenal or submaxillary glands. The nuclei of the injured cells varied considerably. Some were swollen while others were shrunken with centrally clumped chromatin. Approximately 5 per cent contained one to three smooth, round, acidophilic staining bodies whose diameter never exceeded half that of the nucleolus. The acidophilic bodies closely resembled the nuclear inclusions described by Wolf and Orton<sup>27</sup> as occurring in human nerve cells in a variety of pathological conditions. Indeed it is doubtful whether they could be distinguished from those represented in Wolf and Orton's Figures 2 and 3. They were seen only in fixed and stained preparations. Failure to observe them in fresh, still living nerve cells by transmitted light, or in the dark-field, may have been due to their rarity or absence in the particular cells examined. Their even outlines and obvious density were incompatible with the idea of formation by the coagulant or precipitant action of the fixative.

### COMPARISON OF EXPERIMENTALLY PRODUCED INCLUSIONS WITH OTHERS IN VIRUS DISEASES

Since much work has been done in this laboratory on nuclear inclusions in nerve cells injured by the viruses of poliomyelitis and herpes, plenty of material was available for comparison. In addition, specimens of pseudorabies, Borna disease, B virus infection,

and rabbit encephalitis sent to Dr. Cowdry by Drs. E. V. Hurst,<sup>28</sup> I. A. Galloway,<sup>29</sup> A. B. Sabin<sup>30</sup> and C. Levaditi, respectively, were examined. A comparison of less value, because it related to nuclear inclusions in other than nerve cells, was made with preparations of the salivary glands of guinea pigs, moles,<sup>31</sup> hamsters<sup>32</sup> and rats,<sup>33</sup> of virus III disease, fox encephalitis,<sup>34</sup> a virus disease of parrots,<sup>35</sup> and many others generously contributed to the departmental collection.

It is easy to point out differences between inclusions caused by the injection of glucose and the true inclusions in virus diseases. Many will already have occurred to the reader. The experimental inclusions form very quickly — within  $1\frac{1}{2}$  hours after the beginning of injection — whereas in virus diseases they develop more slowly. For example, in pseudorabies they begin to appear 16 hours after virus inoculation (Hurst<sup>28</sup>), and in herpes they are reported after 24 hours (Goodpasture and Teague<sup>36</sup>).

There is a striking uniformity in the experimental inclusions not exhibited by those of the virus diseases. In any particular specimen they tend to be similar in all cells of the same type in contrast to the diversity in phases of nuclear inclusion formation often encountered in neighboring cells afflicted by viruses. In virus diseases it is not a case, simply, of nuclear response to some agent spread impartially by the blood stream to cells of the same kind, for there is local increase of virus in or near particular cells or groups of cells.

The nuclear modifications produced experimentally resembled the *early* changes in certain virus diseases (pseudorabies and rabbit encephalitis) in that the nuclei containing the inclusion bodies showed considerable swelling and a wrinkling of the nuclear membrane. When most conspicuous — from 1 to 2 hours after injection — the experimental inclusions are comparable with those of virus etiology in being separated from the nuclear membrane by a clear halo of material which does not color with acid or basic dyes; but the composition of the experimental inclusions themselves is not exactly the same as that of virus inclusions. Thus, in pseudorabies, herpes, B virus infections and rabbit encephalitis, the nucleoli are but slightly modified, retain their central position and are more or less obscured by the accumulation of material about them.

The material, which clumps about the centrally placed nucleolus to form the experimental inclusions, is irregularly particulate. Never has it been seen to be made up of particles as uniformly granular as

those in virus diseases (yellow fever, for instance). Chemically, also, there seems to be a difference. In the experimental inclusions the material comprises some basophilic (or amphophilic) as well as acidophilic particles. In virus diseases, on the other hand, all or nearly all of the basophilic particles shift to the nuclear membrane against which they marginate, leaving the inclusion wholly or almost entirely acidophilic. The intranuclear inclusions in the salivary glands of moles do stain with basic as well as acid dyes but they are exceptional.

These inclusions, caused by glucose injections, are very temporary modifications. The nuclei soon regain their normal structure. But in virus diseases nuclei possessed of inclusions are apparently on the way to inevitable death and degeneration. The experimental inclusions are not visible in fresh, unfixed preparations, whereas those in yellow fever and herpes are clearly visible with the aid of good optical equipment. This means that in the still living Purkinje cell nuclei some modification has taken place that leads to a different response to fixatives than would occur otherwise. Perhaps the density, or the coagulability of the perinucleolar nucleoplasm has been increased, but this is pure speculation.

The acidophilic masses, found in the nuclei of anterior horn and spinal ganglion cells of distilled water-injected cats, are less definite modifications and perhaps of little significance. They fall in Cowdry's<sup>37</sup> class of type B inclusions (which he says are not so definitely associated with virus action as type A inclusions, of which herpes is the leading example), because they usually occur in a nucleoplasm which shows no other noticeable change and there is certainly no evidence of margination of basophilic chromatin. In their size, arrangement, staining reaction and number they approximate closely the nuclear inclusions in poliomyelitis, as illustrated in Hurst's<sup>38</sup> Figures 4-15. By the same features they resemble the nuclear inclusions in Borna disease (equine encephalomyelitis<sup>39</sup>), but correspond rather more closely to the figures presented by Nicolau, Dimangesco-Nicolau and Galloway<sup>29</sup> than those of Hurst, for the nuclear inclusions illustrated in the former have the appearance of being rather more granular and less hyaline than in the latter. The experimentally induced inclusions were not supplied with halos. It is to be noted that the nuclear inclusions in these two diseases have not been reported in living cells, possibly because they are, like the

artificial ones, not very numerous. Though enumerating these similarities, it is unsafe to suggest identity in respect to the mechanism, osmotic or otherwise, of production. There may be in fact a real difference, since the poliomyelitis and Borna disease inclusions require days to develop, while the experimental ones form in 2 hours or less, the animals always dying during injection of the distilled water. The last named are suggestive of small acidophilic nucleoli, or plasmosomes. However, one would not look for nucleoli to appear so quickly. No explanation is offered as to why they were not produced in the Purkinje cells, like the centrally placed clumps resulting from the injections of hypertonic solutions.

### DISCUSSION

The most obvious initial alteration in the glucose-injected animals is a slight decrease in nuclear volume. This is followed by an increase in volume and a final return to about the original size. The time relations of these changes are presented in Table II. White and Erlanger<sup>40</sup> demonstrated that the immediate effect of injecting a solution consisting of 18 per cent glucose and 25 per cent acacia was a marked increase in blood volume, which slowly returned to normal. Their blood sugar figures show that the excess of glucose is completely removed from the blood stream, and presumably from the tissue fluids, within a period of 2 hours. This time is not very different from the lapse of 4 hours required for the nuclear substance to return to its previous physical state after the injection of 50 per cent glucose. A delay is to be expected in the adjustment of altered nucleoplasm to the reestablishment of normal conditions in the blood because the nucleoplasm is shielded by nuclear membrane, cytoplasm, cell membrane and tissue fluid.

Dehydration of the cell by exosmosis seems to alter the nuclear material in such a way that it is more readily acted on by coagulants. The addition of a minute amount of 0.1 per cent acetic acid causes the fresh nuclei of glucose-injected animals to assume the typical picture observed in fixed material. Lewis,<sup>41</sup> in her work on cultures of connective tissue from chick embryos, observed that by adding small amounts of each of several acids (including hydrochloric and acetic of pH 4.3) tiny granules were produced in nuclei previously appearing homogeneous. When this "gelation" was allowed

to continue the granules became progressively larger and finally a coarse reticulum with occasional larger masses resulted. She makes no mention of a change in the size of the nucleus. When the acid was washed off the nucleus returned to normal but, if the process was repeated several times, the cells soon died. Evidently the concentration of acid in the fixing fluid employed in these experiments was much stronger than that used by Lewis in the reversible gelation of tissue cultures. Substitution, in my experiments, of a fixative devoid of acid resulted in less pronounced granular aggregates in the nuclei. If Lewis had fixed, sectioned and stained cells whose nuclei had undergone gelation there is a chance that nuclear modifications, more like those caused by glucose injection, plus the technique, would have been formed.

Of particular interest is the distribution of the nuclear changes that took place in the cerebellum following glucose injection. They were limited to the nuclei of the Purkinje cells. The smaller and more chromatin-rich nuclei of the granule cells were not altered; nor were the nuclei in the other organs examined (liver, submaxillary gland, testis, ovary, adrenals, and so on), although viruses are known to produce typical inclusions in them. It seems possible that this difference in reaction may be due to the difference in amount of basophilic chromatin, relative to nuclear volume, possessed by chromatin-poor and chromatin-rich types of cells. An interesting parallel, with the viruses, is the fact that no typical virus inclusions have been described in lymphocytes, which possess an abundance of basophilic material, though they are formed in other types of nuclei which contain less.

Weed and McKibben <sup>42</sup> found internal changes, recognizable histologically, in the brains of animals that had been given intravenous injections of hypertonic solutions and that had not been trephined. There was a marked clear space about the nuclei of many cells. The nuclei appeared condensed, the chromatin aggregated. But in animals whose skulls had been opened so that the brains could undergo changes in volume, these histological changes were not demonstrated. For this reason the authors conclude that the alterations are due to increased intracranial pressure, stating however: "That there may be in these brains fundamental histological and cytological differences not revealed by the methods employed, is probable, but further work is necessary to establish such differ-

ences." That the intracranial pressure was not the factor causing nuclear modification following intravenous administration of glucose is evident, because similar changes were produced in the nuclei of sympathetic ganglion cells (Fig. 11) which are not boxed up in an unyielding bony case. They are to be seen also in spinal ganglion cells (Fig. 12).

The possibility that the earlier nuclear changes in pseudorabies, herpes, B virus and rabbit encephalitis are associated with the loss of water from the nucleus is appreciably strengthened by their similarity to those produced by the intravenous injection of hypertonic solutions. The early nuclear changes in Rift Valley fever and yellow fever are likewise accompanied by a loss of water. Findlay<sup>43</sup> noted that, in Rift Valley fever, with the growth of inclusions the nuclear membrane becomes crumpled and shrunken as if fluid had been lost by osmosis. The illustrations of Cowdry and Kitchen<sup>44</sup> apparently show the same phenomenon in experimental yellow fever in monkeys.

The change in the nucleoplasm of large nerve cells, evoked by the intravenous injection of hypertonic solutions and which is indicated after fixation and staining by the appearance of these interesting intranuclear masses, takes place promptly and lasts for only a short time after the stimulus is removed. It very definitely falls short of the production of well formed inclusions characteristic of virus diseases and accompanied by disappearance of nucleoli. But the action of most viruses in the production of inclusions is not systemic; rather is it local and persistent, at least for a period of days. These experiments give no answer to the question whether a more enduring osmotic stimulus, focussed on limited cell groups and supplemented perhaps by cellular injury, which is sometimes thought<sup>44</sup> most helpful in enabling a virus to take hold, would actually lead to the formation of more satisfying intranuclear inclusions.

#### SUMMARY

Two types of nuclear changes were produced: (1) by the intravenous injection of a hypertonic solution of either glucose, glucose plus acacia, sodium chloride or bicarbonate, and (2) by injection of a large quantity of distilled water. Changes of the first type were noted in the Purkinje cells of the cerebellum, anterior horns of the



spinal cord, spinal and sympathetic ganglia and in the pyramidal cells throughout the thickness of the cerebral cortex; while those of the second type appeared in cells of the anterior horns of the spinal cord and spinal ganglia. No nuclear alterations were noted in any other tissues examined.

Nuclear reactions produced by the hypertonic solutions resembled the early alterations in nerve cells following pseudorabies, herpes and B virus infection, while those produced by distilled water more closely resembled inclusions of the poliomyelitis and Borna disease type; but it was not possible to produce any nuclear inclusions which were so much like inclusions in virus diseases that they could be mistaken for them.

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## DESCRIPTION OF PLATES

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### PLATE 25

Photomicrographs at a magnification of 2200 diameters of Zenker-fixed and hematoxylin and eosin-stained sections from the cat's cerebellum, which illustrate the distribution of nuclear chromatin in Purkinje cells of apparently normal animals and in those killed at various intervals after injection of glucose.

FIGS. 1, 2 and 3. Illustrating the characteristic distribution of chromatin particles in control sections.

FIG. 4. Compact mass of nuclear substance enclosing the nucleolus from an animal killed immediately after glucose injection.

FIG. 5. In cats killed 1 hour after injection the nucleus is slightly swollen. The chromatin mass forms a less compact mass about the nucleolus than in Figure 4.

FIG. 6. Showing a greatly enlarged nucleus enclosing a shrunken chromatin mass from an animal killed 2 hours after injection.

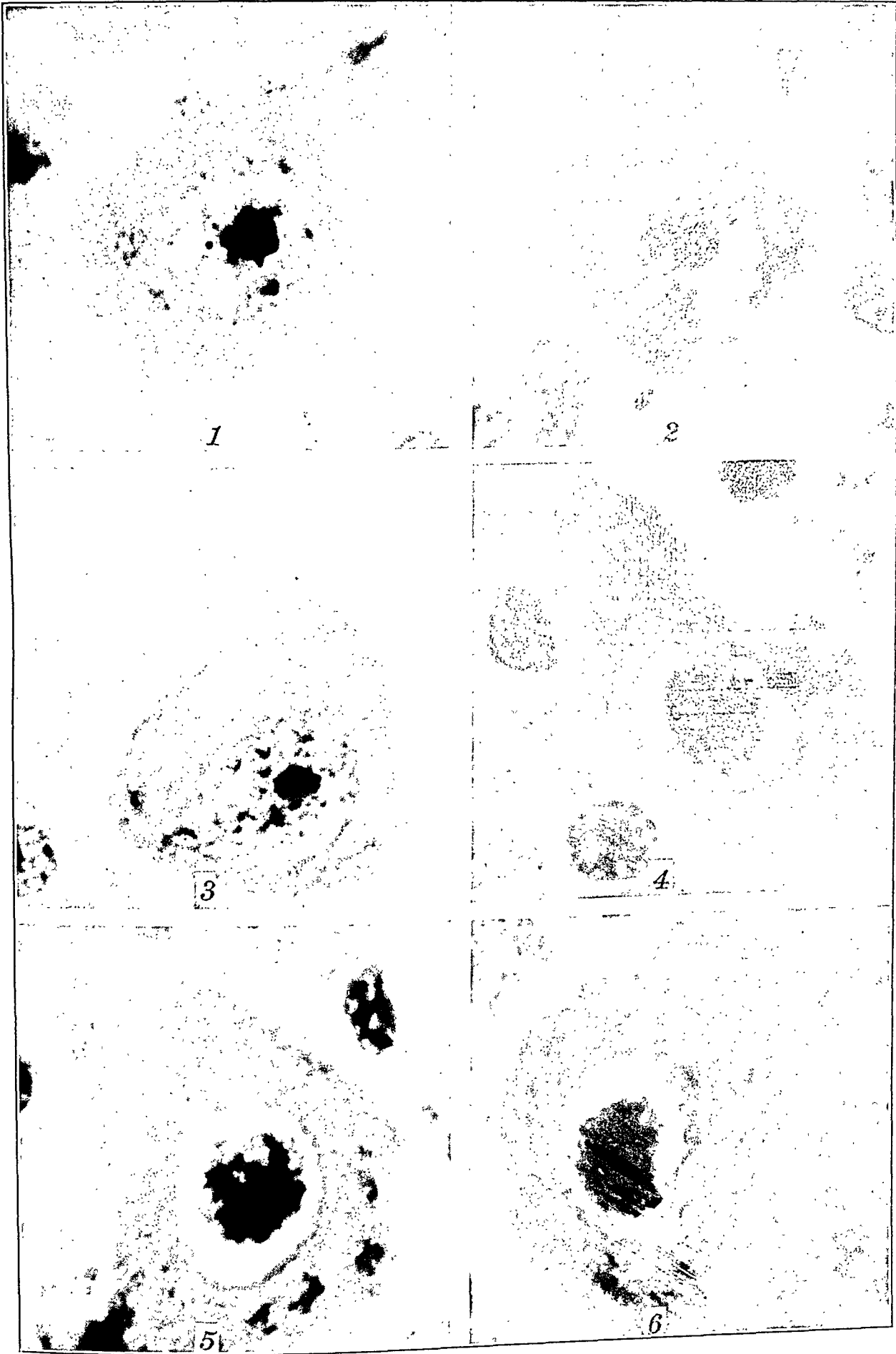


PLATE 26

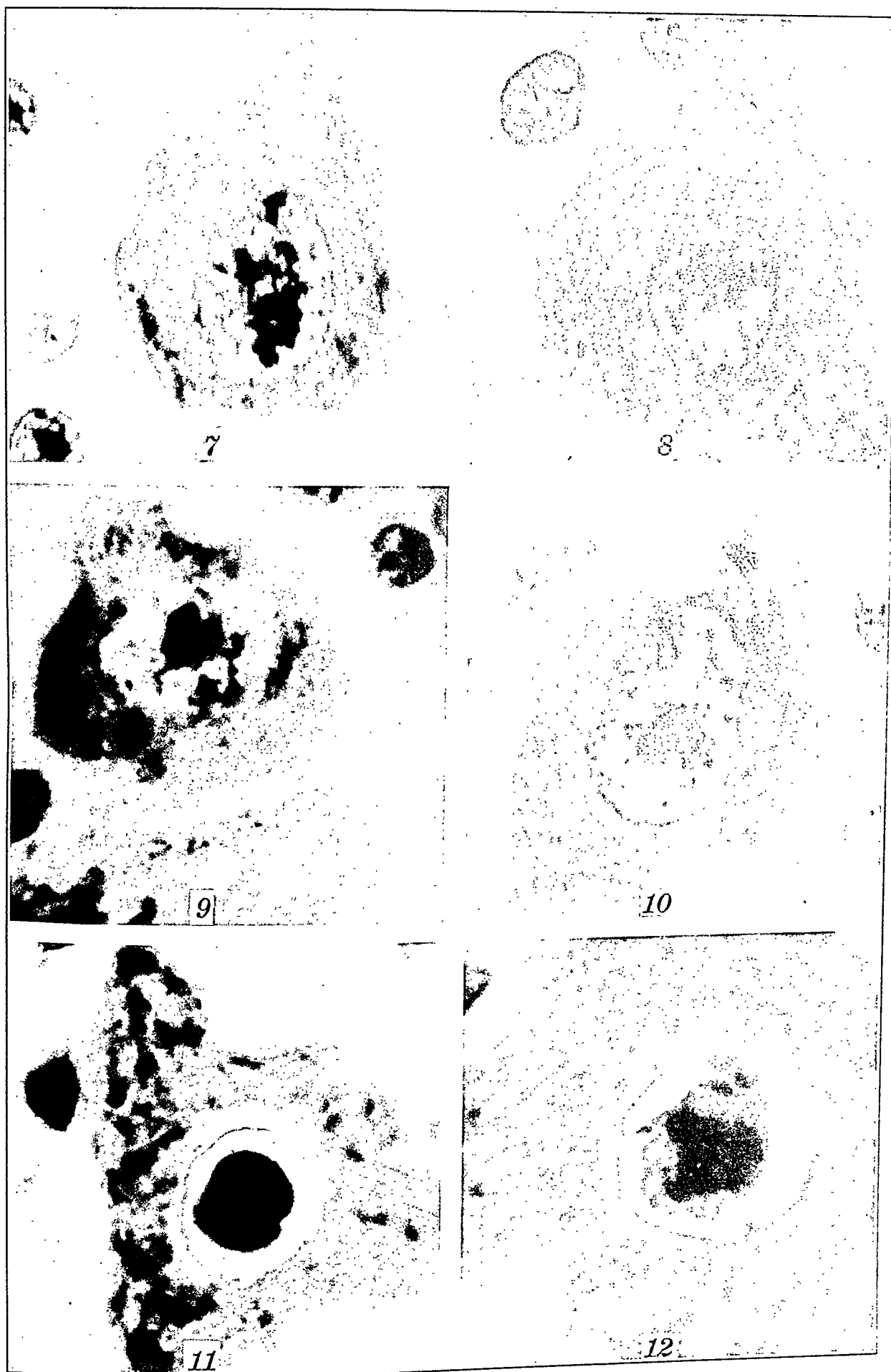
Photomicrographs at a magnification of 2200 diameters of Zenker-fixed and hematoxylin and eosin-stained sections of cerebellum, spinal and sympathetic ganglia from glucose-injected cats.

FIG. 7. Purkinje cell of the cerebellum from an animal killed 3 hours after injection. The nuclear membrane has partially collapsed, obliterating the "halo" seen in Figures 4, 5 and 6.

FIGS. 8, 9 and 10. Purkinje cells from the cerebellums of cats killed 4 hours after injection. A comparison of these with Figures 1, 2 and 3 shows that the distribution of nuclear chromatin is practically the same as that of the control sections.

FIG. 11. Sympathetic ganglion cell which shows shrinkage of nuclear substance when animal is killed immediately after injection.

FIG. 12. Shows shrunken nuclear mass in spinal ganglion cell of a cat killed immediately after injection.





# DERMATOMYOSITIS \*

## A REPORT OF TWO CASES WITH COMPLETE AUTOPSY

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Dermatomyositis was originally described by Wagner in 1887 and almost simultaneously by Unverricht and Hepp. Steiner, in his thorough study, defined the disease as "an acute, subacute, or chronic disease of unknown origin characterized by a gradual onset with vague and indefinite prodromata followed by edema, dermatitis and a multiple muscle inflammation." Although the condition is a rare one, there have been about 85 cases described to date; many of these have not been confirmed by pathological study. The frequency of this condition is probably greater than is apparent, since it is a disease that may readily be overlooked, not only by the clinician but by the pathologist who does not examine voluntary muscle as frequently as he might in general autopsies.

### CASE REPORTS

CASE 1. G. B. (N. I. 12986), a white male, 53 years of age, was admitted to the Neurological Institute in June, 1932. Three months before admission he developed a dermatitis which began with redness and itching about the finger nails. It spread to the arms and was associated with a burning sensation and scaling. Within a week the forehead, eyelids, nose and ears had become similarly affected. A sensation of warmth and itching was particularly noticeable in the skin lesions at night. Two and a half months before entry to the hospital he developed weakness of the arms and legs which was preceded by slight pains in the extremities. The neck muscles became involved so that he had difficulty in holding up his head. This weakness grew progressively worse and he had to stop working  $1\frac{1}{2}$  months before admission. He next began to suffer from difficulty in speech and swallowing, frequently regurgitating food through the nose. Night sweats appeared and the skin became dry and painful over the buttocks and back. He was confined to bed during the month before admission, any exertion causing him to become dyspneic. The dysphagia increased and he had difficulty in coughing up the large amounts of mucus that collected in his throat. There was a sense of tightness in the skin of the arms and legs. There had been a loss of 5 to 6 pounds during the 3 months of illness.

\* Received for publication September 23, 1935.



On admission the patient appeared chronically ill. The temperature was 99.4° F., the pulse rate 96 per minute, respiratory rate 24 per minute and blood pressure 136/68. There was a red, dry, scaling pruritic rash present on the forehead, eyelids, ears, chest, neck and extensor surfaces of the arms and legs. There was extreme muscular weakness with considerable atrophy involving all the muscle groups. Dyspnea and dysphagia were present, and he could not cough effectively. The blood count showed a hemoglobin of 82 per cent, 4,300,000 red blood cells and 24,000 white blood cells per cmm., with 85 per cent polymorphonuclears, 10 per cent lymphocytes, 4 per cent large monocytes and 1 per cent eosinophiles. The urine contained a few white blood cells, but was otherwise negative. The spinal fluid findings were negative. There was no edema and the spleen was not enlarged.

Four hours after admission the patient's temperature rose to 104° F., although no new symptoms appeared and no new focal signs were found. The next morning he was somewhat better. His temperature was 102° F., and he was coughing less and swallowing more easily. Just after finishing luncheon, without any apparent difficulty, he suddenly became cyanotic, coughed a good deal, lapsed into stupor and died a few minutes later. No definite diagnosis was made.

### *Postmortem Examination*

#### *Gross Findings*

At autopsy an extensive rash was noted over both forearms, arms and shoulders. The skin in these areas was scaling, roughened and slightly injected. It felt firm and was fixed to the underlying tissues. The eyelids were extensively involved in the same process, being covered by thick, dry white scales. There was no pitting edema. The thorax, abdomen and lower extremities were entirely free of skin lesions. All the skeletal muscles were very pale, being light grayish in color, and seemed normal in consistence. There did not appear to be any gross increase in fibrous tissue in them and no replacement by fat. The other positive findings were as follows:

A few localized fibrous adhesions were present in the region of the cecum and when these were separated about 5 cc. of thick, greenish yellow pus were disclosed in the retrocecal position lying directly posterior to the shortened, thickened and slightly injected appendix. The process was well localized.

The left pleural cavity contained about 150 cc. of cloudy yellow fluid. There was a delicate fibrinous exudate over the pleural surface of the lower lobe of the left lung. The bronchi of this lobe contained purulent material. There were many irregular areas of consolidation in the parenchyma.

The gall-bladder contained about fifty hard, jagged gall-stones.

Its mucosal surface was smooth and deeply bile-stained. At the extreme apex a rather hard nodule, covered by mucosa and measuring 1.5 by 1 by 1 cm., protruded into the lumen. On section it was finely granular and yellowish gray. It extended through the muscular coat but had not penetrated the serous covering. A similar nodule, measuring 2 cm. in diameter, apparently in a lymph node, lay adjacent to the cystic duct, which was patent.

The brain, spinal cord and meninges showed no gross abnormalities, externally or on section.

### *Histological Findings*

*Skin:* On the surface of the skin there was a thick layer of cornified material containing considerable nuclear and some eosinophilic granular debris. The epidermis was considerably compressed and flattened over large areas and here the normal papillae were not in evidence. The basal cells of the epidermis showed marked vacuolization of their cytoplasm. No pigment was noted in the epidermis and only occasional, small pigmented cells were found in the corium. The dermis was thickened and its collagen masses appeared considerably swollen and somewhat hyaline. Just beneath the epidermis there were scattered lymphocytes, polymorphonuclear and large mononuclear leukocytes. There was a mild perivascular exudate of the same type, chiefly about the vessels in the outer portion of the corium. A similar infiltration was seen about the ducts of the sweat glands.

*Muscle:* Scattered perivascular infiltrations by lymphocytes, large mononuclears, plasma cells, a few polymorphonuclear leukocytes and occasional mast cells were present. There was diffuse infiltration as well. These infiltrative cells were found chiefly in the perimysium but were present, scattered, in the endomysium as well. Quite a number of muscle fibers showed loss of their transverse striations. Some fibers stained faintly and presented vacuolated zones which contained varying amounts of finely granular, eosinophilic material. Frequently there was a considerable multiplication of the muscle nuclei. The sarcolemma sheaths were occasionally raised over small areas, producing surface blebbing.

In the more severely affected muscles the perimysial infiltration was often quite intense. A great many muscle fibers were pale stain-

ing and showed loss of their striations. They were vacuolated and exhibited fragmentation of their fibrils. Frequently they were partially hyalinized. A considerable increase in the perimysial connective tissue was observed and some multiplication of sarcolemma cells. Fibrous replacement of degenerated muscle fibers had occurred and this fibrous tissue was at times diffusely infiltrated. Occasionally the sarcolemma sheaths appeared to be torn and muscle fibrils protruded through the tear. Irregular multinucleated giant cells lying in the course of degenerated muscle fibers were seen. These had a somewhat more bluish tinge to their cytoplasm than the neighboring muscle fibers and their nuclei contained more chromatin. They appeared to be an attempt at muscle regeneration. Considerable, finely globular neutral fat was seen in the degenerating muscle fibers. The small nerves in the muscle showed occasional perineurial infiltration similar to that in the perimysium, but no other changes. The muscle spindles appeared normal.

*Other Tissues:* Representative sections of the cortex, basal ganglia, brain stem and spinal cord revealed no abnormal histological findings.

The nodule in the fundus of the gall-bladder proved to be an early carcinoma of that organ. The mass in the lymph node near the cystic duct was the only metastasis.

The appendix exhibited the results of a suppurative inflammation and on its surface there was granulation tissue forming the wall of the periappendiceal abscess.

The lung showed a terminal lobular pneumonia.

CASE 2. M. M. (P. H. 377533), a white female, 47 years of age, was admitted to the Presbyterian Hospital on May 10, 1933. The initial symptom was a dermatitis which developed 8 months before admission, beginning on the palms. The skin at first was leathery in consistence; later, a generalized, red scaling eruption developed and the skin became thickened. This was diagnosed as dermatitis exfoliativa. She began to lose weight. Three months later the dermatitis began to clear up. Eight weeks before admission difficulty in articulation and swallowing developed. A right peritonsillar abscess was discovered at about this time and 4 cc. of foul pus evacuated from it. During the last 3 weeks before admission the patient grew progressively weaker. The impairment of speech and swallowing became more intense. She coughed frequently and ineffectively, much mucous secretion collecting in her throat. Weakness of the legs and back developed. She was able to take only fluids and had lost 68 pounds since the onset of illness.

On admission the patient appeared chronically ill and showed evidence of a loss of weight. Her speech was unintelligible, accomplished with considerable

exertion, and followed by marked fatigue and dyspnea. There was mucus in the throat and trachea. Only the cervical lymph nodes were palpable. The lungs, heart and abdomen were negative. There was no clubbing of the fingers, no cyanosis and no edema. The skin eruption had entirely cleared save for some residual thickening, especially on the feet, and a little scaling at the elbows. Deep reflexes were diminished. The diaphragm, intercostal and abdominal muscles were weak. The temperature was 99.2° F. on admission, rose to 100.6° on the 2nd day and then returned to normal, to remain there. Blood pressure was 110 systolic and 80 diastolic. The pulse rate varied from 94 to 102 and respiratory rate from 22 to 40 per minute. The hemoglobin was 94 per cent, red blood cells 4,900,000 per cmm. and white blood cells 13,700, with 80 per cent polymorphonuclear and 8 per cent mononuclear leukocytes, 1 per cent transitional forms and 1 per cent lymphocytes. The Wassermann test was negative in the blood and spinal fluid. The spinal fluid was not abnormal. A neurological examination revealed no sensory changes. There was weakness of the palate and an impaired swallowing mechanism. The vocal cords were not paralyzed. The intercostal, diaphragm and abdominal muscles and the extensor quadriceps muscle groups were weak.

The patient had increasing difficulty in breathing and swallowing. She was placed in a respirator but failed to improve. On the 4th day in the hospital she became irrational and maniacal and died after several hours of excitement. The clinical diagnosis was not definitely established, but was thought to be bulbar paralysis terminating a poliomyelitis.

### *Postmortem Examination*

#### *Gross Findings*

At autopsy the body was found to be well developed and in fair nutrition. Moderate scaling and thickening of the skin of the neck, hands, forearms, elbows and soles of the feet (most marked on the latter) were present. There was no evidence of edema. The temporal, pectoralis minor and intercostal muscles were very pale and grayish pink. The psoas muscles, right rectus femoris and muscles of the back were also paler than normal, but the remainder of the skeletal muscles examined appeared grossly normal. The rest of the gross examination was essentially negative save for a moderate degree of congestion of the viscera. The central nervous system, brain and spinal cord, revealed no gross abnormalities.

#### *Histological Findings*

*Skin:* Hyperkeratosis was present. The outermost cell layers contained pigment. The vessels of the corium showed a mild perivascular lymphocytic infiltration.

*Muscle:* An intense inflammatory reaction was observed in the perimysium and to a lesser extent in the endomysium. The cells

TABLE I  
*Analyses of Muscle*  
(Case 2)

Muscle	Moisture	Creatine		Total nitrogen		Ratio creatine N <sub>2</sub>
		Wet	Dry	Wet	Dry	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Right rectus femoris	79.64	0.098	0.482	...	...	...
Left pectoral .....	76.37	0.255	1.077	2.85	12.08	2.86
Right psoas .....	81.47	0.253	1.365	2.65	14.30	2.48
Right back muscle .	75.86	0.109	0.452	...	...	...
Left rectus .....	74.82	0.283	1.125	...	...	...
Left intercostal ....	71.64	0.136	0.480	...	...	...
Diaphragm .....	78.25	0.070	0.322	...	...	...

*Case of Dermatomyositis Cited by Steinitz and Steinfeld*

Diaphragm .....	0.282	Different parts of same muscle
Adductor femoris .....	0.310	
Biceps .....	0.188	
	0.143	

*Normal Cases (Adults) Cited by Bodansky*

Diaphragm .....	0.331 0.309 0.309	3 different cases
Myocardium .....	0.261 0.257 0.220	"
Psoas .....	0.485 0.465 0.448	"
Intercostal .....	0.220? 0.266?	2 different cases (difficult to separate muscle fibers)
Pectoralis major .....	0.447 0.456 0.433	3 different cases

consisted mainly of lymphocytes accompanied by a few plasma cells and eosinophiles and many mononuclear cells. The myofibrils could be seen in many of the muscle fibers, but the cross striations were often indistinct. Some fibers showed hyaline and others vacuolar

degeneration. Granular debris was present in many of the vacuoles. Frequently there was an increase in the muscle nuclei, possibly a condensation, as well as in the sarcolemma sheath nuclei. Many of the muscle fibers appeared to be fragmented and their ends were retracted and coiled. In a few areas there were groups of large, elongated mononuclear cells with fibrils which were probably myoblasts. Some of these cells showed mitoses.

The changes were severe in the intercostal and back muscles examined, as well as in the tongue. They were moderate in the rectus femoris, psoas, rectus abdominis and temporal muscles.

No microscopic abnormalities were noted in representative sections of the brain and spinal cord.

Analyses of various muscles obtained at autopsy were made by Dr. Goettsch and the accompanying table gives the results. For comparison the results of a similar chemical study by Steinitz and Steinfeld are added, as well as Bodansky's figures (see Steinitz and Steinfeld) obtained in normal human muscle. The creatine content is observed to be lowered in the diseased muscles. The degree of this diminution appears to correspond roughly to the extent of involvement of the muscles.

### DISCUSSION

Dermatomyositis occurs equally in both sexes and is more frequent in middle life. A little less than one-third of the reported cases, however, occurred in the first two decades. The disease is more common in the temperate zone and there is no definite seasonal incidence, although possibly its inception is more frequent in the winter months.

The clinical picture may be quite variable. There may be an initial period of malaise, generalized pains, insomnia and anorexia, or the disease may begin with acutely painful muscles, usually in the extremities, but in other cases the skin lesion may occur first. The muscles become swollen, painful and definitely weak. There is no rule as to the areas first involved, but often the upper extremities, shoulders and face are the primary site. The entire voluntary musculature as well as the muscles of deglutition and respiration may be involved eventually. A doughy edema of the skin is present which may produce a considerable increase in the size of the extremities and a mask-like facies. The skin lesion may consist of an erythema re-

sembling an erythema nodosum or multiforme, or the skin may appear tight and glistening. The skin lesions, however, may vary considerably in type. The mucous membranes may be involved in certain cases, producing a stomatitis with ulceration or an angina. There is usually a moderate fever, and not infrequently an enlarged spleen, with a tendency toward profuse perspiration. As the disease progresses, muscular weakness becomes more marked and movement, both active and passive, impaired. The muscles of respiration and mastication may become involved and dysphagia and respiratory embarrassment occur. This may lead to asphyxia or an aspiration pneumonia. The mortality is fairly high, approximately one-half the cases ending fatally. Of the 28 cases collected by Steiner, 17 were fatal. Those cases that tend to recover may run a chronic course and the marked muscular damage may lead to atrophy and fibrosis, contractures, and even to calcification. Residual changes may be noted in the skin which remains thickened and often pigmented. This cutaneous change frequently resembles that of scleroderma, and Klingman and others suggest that scleroderma may be preceded by this condition. Occasionally the disease has an intermittent character. It may be fatal in from 1 to 4 weeks or death may occur after nearly 2 years. Recovery or death usually takes place in a few months.

Clinically the 2 cases reported here showed some atypical features. In neither of the 2 cases was pain an outstanding symptom. The pain in this disease is of course due to the myositis. That there were widespread inflammatory lesions of the musculature in both our cases is certain from the pathological examination. It may be that the intensity of the pain in other instances of the disease is dependent on the degree of involvement of the intramuscular nerves. This was relatively slight in both of our cases.

In each instance the dermatitis apparently preceded the myositis and neither patient at the time when in the hospital exhibited any of the doughy edema described in other cases. It may be that this was present earlier in the disease, although no clear history of it was obtained from either patient. The edema was slight in Jacoby's case (see Steiner). The leathery, tight, somewhat shiny skin adherent to the underlying tissues so consistently reported as a late stage of the skin lesion in dermatomyositis was present in both of our cases.

In the first patient considerable muscular atrophy was present. This has been mentioned as a sequel of the disease, but not sufficiently indicated as a possible part of the syndrome. It is obvious from histological study of the musculature that there is widespread degeneration of muscle fibers with a consequent loss of muscle substance. When the process is a very rapid one, as it was in the first case, it is understandable that a considerable muscular atrophy may develop quickly.

### *Pathology*

The disease process is an inflammation, commonly with degeneration, of the skin and striated musculature including the muscles of deglutition and respiration. Although the disease is most conspicuous in the skeletal musculature, in a small percentage of cases the heart muscle is also involved. Smooth muscle fails to develop the lesions.

Grossly the skin lesions may be quite variable in character. They may appear as an erythema, erythema nodosum, erythema multiforme, erysipeloid lesion, roseola or urticaria. They are most frequently accompanied by a non-pitting edema. Later the skin may scale and become brownish, leathery, tight, shiny, atrophic and attached to the underlying tissues. As mentioned above, the end result may be a scleroderma. Microscopically the skin shows flattening of the papillae and vacuolization of the cells in the basal layer. The dermis is edematous and the collagen fibers appear swollen and semihyaline. Mild round cell accumulations occur about the vessels and sweat glands. These cells are chiefly lymphocytes and monocytes and the infiltration is most common directly beneath the epidermis. Later there is desquamation of considerable cornified epidermis with thinning of the latter, the papillae may disappear over large areas and there is a widespread increase of collagen in the corium. The underlying fatty tissue secondarily affected by the inflammation may undergo some fibrosis.

Grossly the affected muscles are dull, grayish yellow, yellow or pale red in color, and firm and rubbery, or soft and doughy. They may have small scattered hemorrhages in them and be friable. Later they show much loss of substance and fibrosis, rarely with calcification. Microscopically the muscles show edema of their endomysium and perimysium with from mild to marked perivascular and diffuse



round cell infiltrations in the same membranes. The inflammatory cells include lymphocytes, large mononuclears, plasma cells, some polymorphonuclear leukocytes and occasional mast cells. In later stages the endomysium and perimysium are increasingly thickened. The muscle fibers show a loss of striation, fragmentation of fibrils, granular, hyaline or waxy degeneration, vacuolization or rupture. There may be marked irregularity in the size and an increase in the number of muscle and sarcolemma nuclei. Frequently there is blebbing of the sarcolemma sheath. Multinucleated cells may be present as evidence of attempts at regeneration.

The etiology of the disease is unknown. Because of the acute onset, the fever, angina, frequently enlarged spleen, sweats and muscle inflammation, it long has been suspected that it is an infection. A great variety of causative agents have been suggested. Unverricht suggested a gregarine; Langsteiner (see Steiner), a streptococcus; Bauer, a staphylococcus; Martinotti, a micrococcus; and others a variety of other organisms, but none of these has been found consistently in the disease. Strümpell called attention to the occurrence of tuberculosis in some of these cases and thought there was a possible relationship. Grunke reported an instance with a typical clinical syndrome in which the lesions were obviously tuberculous. The disease has been observed following upper respiratory infection, acute rheumatic fever, some of the acute infectious diseases of childhood and gastro-intestinal intoxications. More recently it was described in the presence of chronic suppurative foci following typhoid by Costanzi. It is possible that more than one agent may either produce this condition or be the predisposing factor. It is interesting that in our cases foci of infection were present; in the first case a peri-appendiceal abscess, in the second a peritonsillar abscess. Gram, methylene blue, carbol-fuchsin and Jahnke stains revealed no organisms. A careful search of our skin and muscle material for cell inclusions which would indicate the possibility of a virus origin of the disease yielded negative results. No fresh material for injection into animals was available.

Although it seems more probable that the condition is an infection, degenerative changes with secondary inflammatory phenomena in the skin and muscles due to toxins from some other primary condition are another possibility. Similar muscle lesions produced in guinea pigs and rabbits by Goettsch and Pappenheimer by the use

of special diets show many features of the muscle lesions of dermatomyositis. No definite evidence, however, of improper nutrition has been encountered in the known cases of this disease.

Steinitz and Steinfeld studied the creatine metabolism in a case of dermatomyositis and found it to be markedly affected. There was a constant creatinuria with a low total creatinine excretion, coupled with an inability to handle orally administered creatine. Some of the muscles showed a decreased creatine content. This was checked in our second case and the creatine content was below normal in each of the seven muscles examined.

In conditions in which muscle tissue undergoes degeneration it is known that creatinine excretion is lowered and creatine appears in the urine. The loss of creatine in the muscle is probably directly dependent upon the amount of muscle tissue destroyed.

Steinitz and Steinfeld refer to the case of "myositis fibrosa" of Bodansky, Schwab and Brindley which they think may have been a late stage of dermatomyositis. The creatine metabolism was studied in that case and the changes observed were similar to those found by Steinitz and Steinfeld in their case, except that there was no diminution in the total creatinine excretion. These chemical studies, although interesting, throw no light on the essential etiology of the disease.

#### SUMMARY

1. Two cases of dermatomyositis are presented with autopsy reports. One was a male, 53 years of age, with a history of 3 months duration, and the other a female, 47 years of age, with a history of 8 months duration.

2. Each case showed widespread inflammatory and degenerative lesions of the skeletal musculature and less disseminated skin lesions. In 1 case there was an acute and in the other a chronic focal infection.

3. Atypical features, such as the slight pain, the small amount of edema and the advanced muscle atrophy in 1 case are pointed out.

4. The results of a quantitative determination of the creatinine content of the affected muscles in 1 case are given.

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## DESCRIPTION OF PLATES

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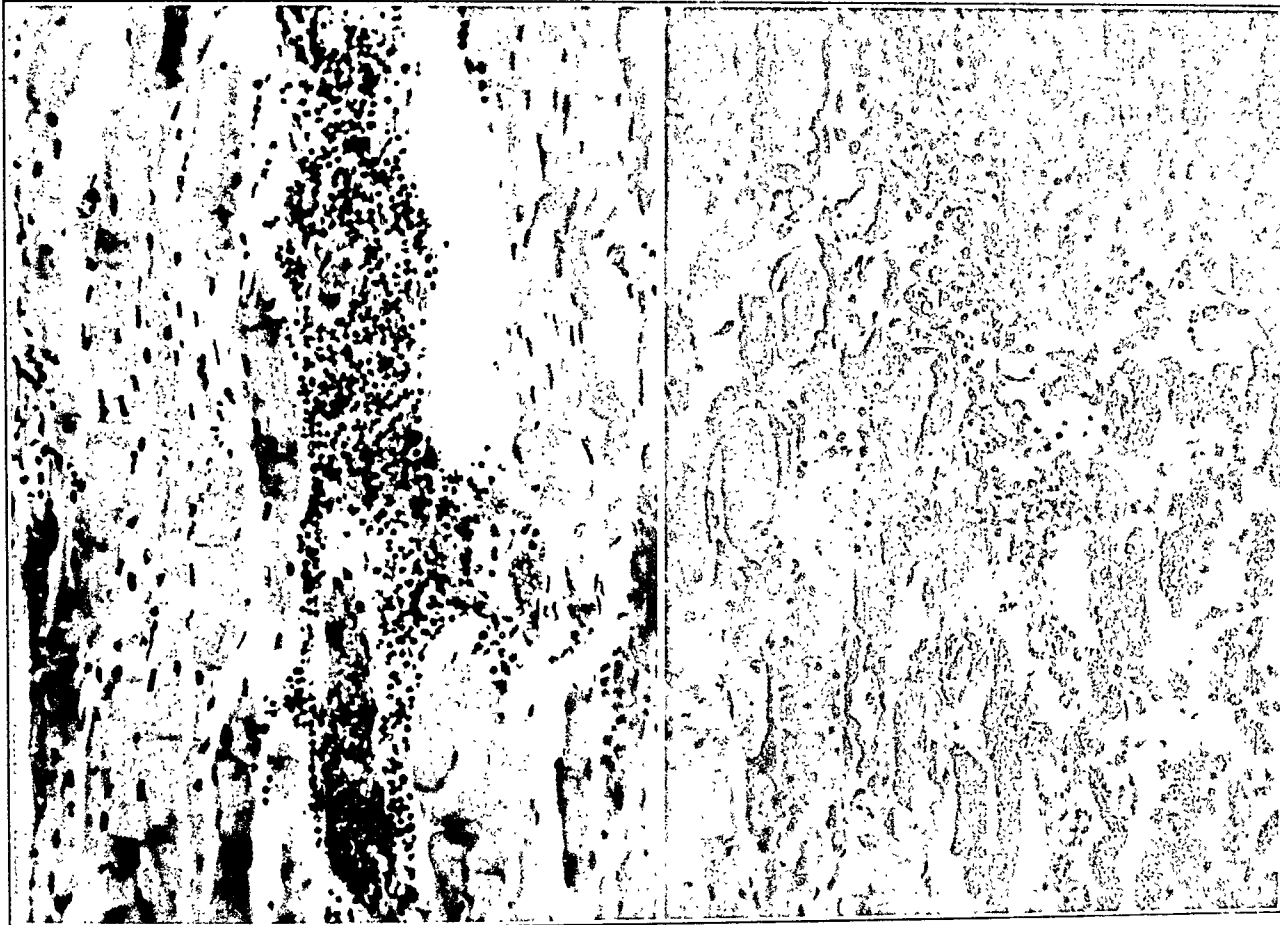
### PLATE 27

FIG. 1. Round cell infiltration of perimysium and endomysium of striated muscle (Case 1).  $\times 100$ .

FIG. 2. Degeneration and disappearance of muscle fibers. Edema and mild, diffuse, round cell infiltration (Case 1).  $\times 100$ .

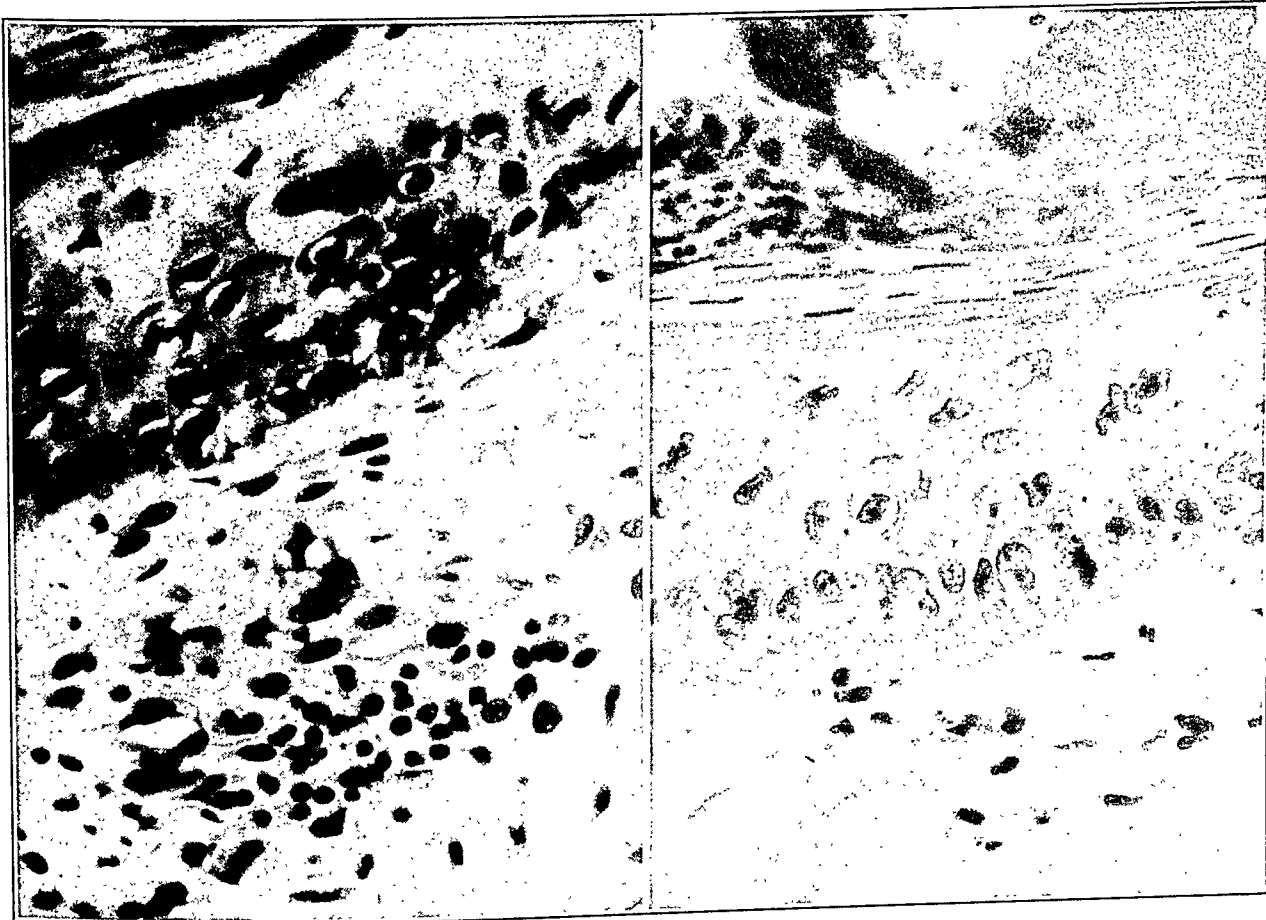
FIG. 3. Round cell infiltration in corium directly beneath epidermis. Flattening of papillae (Case 1).  $\times 460$ .

FIG. 4. Vacuolization, especially of basal layer of epidermis. Edema of corium (Case 1).  $\times 460$ .



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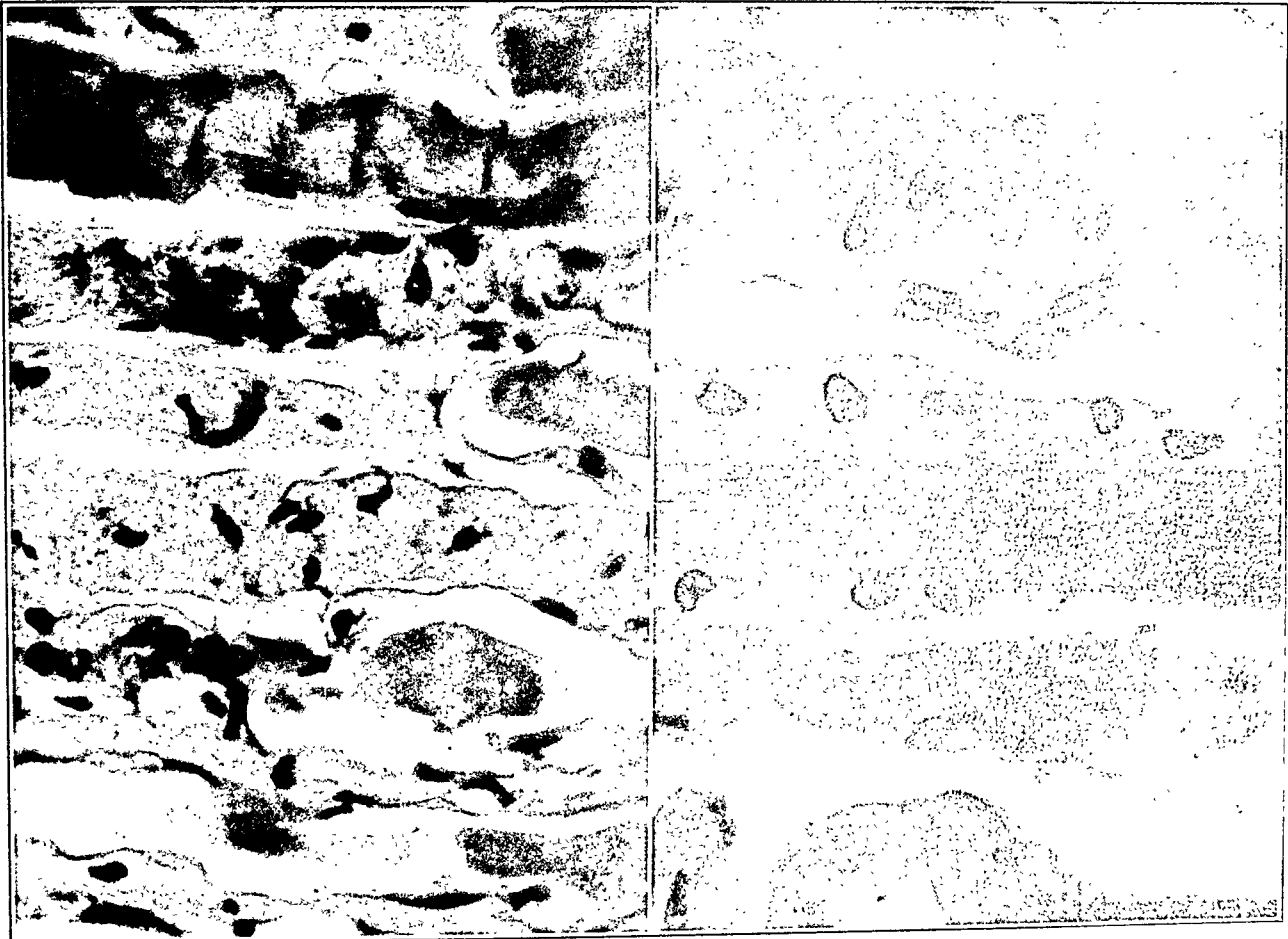
PLATE 28

FIG. 5. Normal muscle fiber at top. Other fibers all undergoing necrosis, illustrating granular, hyaline and vacuolar degeneration. Increase of muscle and sarcolemma nuclei (Case 1).  $\times 460$ .

FIG. 6. Blebbing of sarcolemma sheaths of muscle fibers (Case 1).  $\times 700$ .

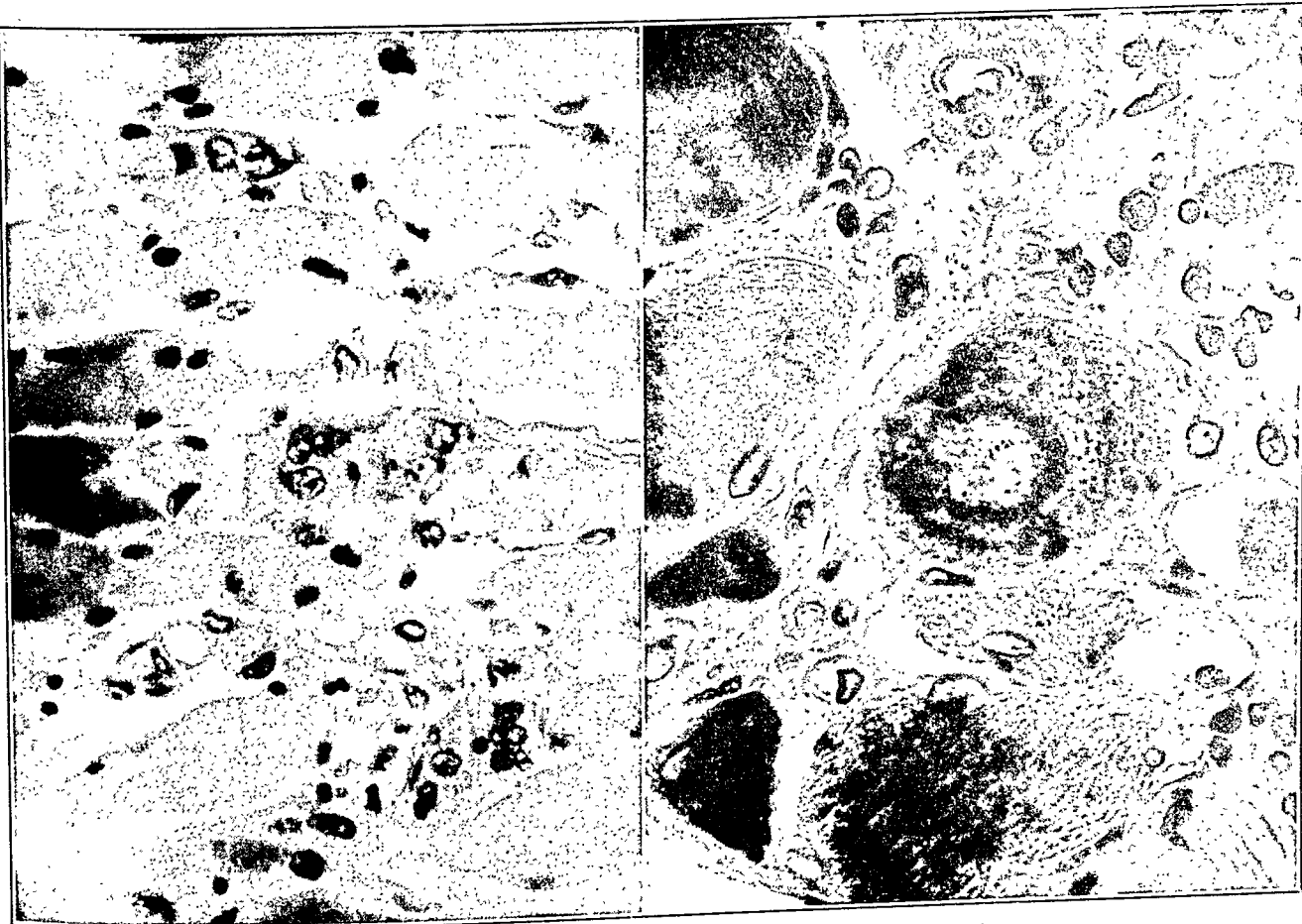
FIG. 7. Multinucleated cells with deeply staining cytoplasm in degenerated muscle. Probable attempt at regeneration (Case 1).  $\times 700$ .

FIG. 8. Cross-section of muscle fiber undergoing degeneration. Thickened endomysium and perimysium. Mild round cell infiltration (Case 2).  $\times 700$ .



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# SPONTANEOUS LEUKEMIA AND CHLOROLEUKEMIA IN THE RAT\*

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Leukemia is known to occur in almost all of the common domestic and laboratory animals. It has repeatedly been described in fowl, cattle, horses, swine, canaries, dogs, cats, mice, guinea pigs and monkeys. The literature on this subject has been reviewed by Opie<sup>1</sup> and by Bayon and coworkers.<sup>2</sup> Apparently the disease has never been observed in rats. This hiatus is all the more surprising when the large number of rats under constant laboratory observation is considered.

Chloroma, or chloroleukemia, an uncommon condition in man, has been infrequently observed in animals. It has been reported in the pig by Claussen<sup>3</sup> and Joest.<sup>4</sup> Hill<sup>5</sup> mentions among the atypical cases in a large group of leukemic mice, 1 in which the lymph glands and tumor-like growths were bright green in color. This was classified as chloromyelosarcoma. Mathews<sup>6</sup> has described a form of fowl leukosis associated with local tumors composed of myeloid cells. He designated this condition as leukochloroma and related it to chloroma in man, although the tumor masses were described as chalky white. The green pigmentation, which has always been considered a prominent feature in this condition and from which the name is derived, was lacking.

The purpose of this report is to record 12 instances of leukemia developing spontaneously in an inbred strain of albino rats. Eleven of these were of the myeloid type and 1 of the lymphatic. Of the myeloid group, 4 showed a very striking light green pigmentation in all areas where leukemic cells were present in abundance and are, therefore, classified as chloroleukemia. The diagnosis of leukemia was based on autopsy findings, the criteria being leukemic changes in the bone marrow, spleen, lymph nodes and blood vessel contents, as well as infiltrations into the viscera.

\* Received for publication October 3, 1935.

The rats, pure albinos of Osborne-Mendel stock, had been bred in the laboratory of Dr. H. C. Sherman of the Chemistry Department, Columbia University, for many generations over a period of years and were subjected to nutritional experiments.\* In no case was the presence of leukemia suspected during life. Blood cell counts were not done.

The total number of deaths due to spontaneous causes in the colony over a period of about 20 months was 365. All the animals were subjected to a complete gross and microscopic examination. Twelve showed clear-cut evidence of leukemia, an incidence of 3.3 per cent. The most plausible explanation for the fairly high incidence of leukemia in this particular group is on an hereditary basis. Richter and MacDowell<sup>7,8</sup> have emphasized the genetic factor in mouse leukemia. They showed that not only was there a marked difference in the incidence of spontaneous leukemia in several strains of mice, but also that mice of a strain with a high incidence were all readily susceptible to experimentally transmitted leukemia, whereas the non-leukemic strains were comparatively refractory.

There is no evidence that leukemia tended to occur in certain families within the strain. There were no litter mates in this group, nor were there any of common parentage, and only two had a single common grandparent. In other words, although the occurrence of leukemia in this stock seems to have its basis in some inherited factor, inasmuch as this condition has not been observed in heterogeneous or other inbred strains, it is impossible to trace a closer familial relationship within the strain itself. Admittedly, the number of cases observed is insufficient to rule out conclusively such a possibility. About 25 litter mates of the leukemic animals have died, most of them with longer life spans, and none has shown any evidence of the disease. It is true that only 5 animals, 2 males and 3 females, were retained for experimental purposes in each litter. This might have obscured any tendency for the disease to appear in individual families, especially as the disease in this particular group was more prevalent in males than in females. There were 8 males and only 4 females.

In addition to the leukemic rats there was another group of 36 in this series of 365 which showed evidence of marked myeloid meta-

\* We wish to acknowledge our indebtedness to Dr. H. C. Sherman, Dr. H. L. Campbell and Dr. L. N. Ellis for making this material available.

plasia and enlargement of the spleen, but no other changes attributable to leukemia. In many the splenic changes simulated leukemia in every respect. The organ measured from 4 to 6 cm. in length. The architecture microscopically was completely obliterated, follicles and venous sinuses being invisible. The pulp was uniformly filled with leukocytes, a large proportion of which were fairly mature, with polymorphic nuclei and neutrophilic granulations in the cytoplasm, readily demonstrated with the Giemsa stain. There were, however, still greater numbers of neutrophilic myelocytes and some eosinophils and megakaryocytes as well. Mitotic figures were numerous.

It is difficult to evaluate this group. It is possible that it includes a few real but very early cases of leukemia, which might have been recognized if white blood cell counts had been available. Possibly the splenic picture represents a preleukemic change. If so, the total incidence of leukemia and potential leukemia is brought up sharply in this strain to 12.2 per cent. It seems more likely that this myeloid transformation is a response to prolonged and widespread pyogenic infection. Females predominated in this group, there being 28. Of these, 23 had extensive uterine infection with the formation of large old abscesses. The entire group showed extensive pulmonary and middle ear suppuration. Evidence of hematopoiesis is almost constantly present in the splenic pulp of this strain of rats. It is not improbable that infection followed by longstanding leukocytosis may have called forth a marked myeloid response in the spleen. It must be admitted that many of the remainder of the animals also showed extensive pyogenic infection of equal severity without, however, any significant alterations in the spleen. No obvious familial relationship, either to each other or to the leukemic animals, was found to exist in the members of this group.

No information concerning the duration of the leukemic process is at hand. The changes in the viscera and the type of cell involved conform in every case to the chronic variety of the disease as it is seen in man. As regards the duration of life, the average age at death of the 4 females was 669 days, as against an average of 801 days for 110 non-leukemic female rats. Three of the 4 died at an age well below that of the average for the female controls. The average life span of the 8 leukemic males was 718 days, only slightly less than the average life span of 100 non-leukemic males, which was 738 days.

Four of the leukemic males died at a younger age and 4 outlived this control average.

It would appear then that the presence of leukemia in these animals did not appreciably alter the duration of life. If anything, they died at a slightly younger age, but 5 of the 12 lived longer than the average of the control groups of the same sex. It will be noticed that the disease was found only in fairly old rats; none was under 18 months of age and the majority were well over 2 years. It is possible that this fact may in part account for the failure to observe leukemia as an incidental finding in the general run of laboratory rats, since the major portion is discarded or sacrificed before reaching the leukemic period.

From the time of weaning all the rats were maintained on diets of known composition. These diets consisted chiefly of ground whole wheat and dried milk powder, in varying proportions, with a few additional constituents such as green vegetables or lean beef. In each instance the diet was of sufficient caloric, protein, mineral and vitamin content to support good growth, nutrition and reproduction. The diets were analyzed to see if any favored the development of the disease. Leukemia developed in animals on six different diets. There was one diet on which 40 animals were fed, none of which developed leukemia. It consisted of: whole wheat 77.44 per cent, whole milk powder 16.41 per cent, butter fat 4.59 per cent, sodium chloride 1.56 per cent. The average life span of the 40 animals was longer for both sexes than that of leukemic ones.

There was a second diet including only 19 animals, 3 of which developed leukemia, an incidence of 15.8 per cent, which is considerably higher than that of any other diet. Its composition was: flour 35 per cent, milk powder 16 per cent, dried beef 10 per cent, lard 7 per cent, whole wheat 6 per cent, butter fat 4 per cent, dried potato 9 per cent, sugar 9 per cent, dried orange juice 4 per cent. The non-leukemic animals on this diet had a shorter average life span for both sexes than the control groups. With the exception of these two discrepancies, both of which are very likely accidental, no dietary influence in the development of leukemia was found. There was certainly no obvious ingredient in the food, the presence or absence of which favored the development of the disease.

The gross features of the disease were as follows: The spleen was

in each case markedly enlarged, measuring from 5.4 to 8.5 cm. in length. In 1 case of chloroleukemia the spleen was tremendous in size, weighing 15.5 gm. and measuring 8.5 by 2.5 by 1.5 cm. It filled more than half of the abdomen. The capsule was usually tense and slightly thickened. The pulp was firm, dark red, non-friable and homogeneous. The lymphoid follicles and trabeculations were indistinguishable. In several cases scattered, irregular, sharply outlined, pale yellow ischemic infarcts had formed. In the chloroleukemia cases the pulp had a slight green cast superimposed on the otherwise grayish red pulp.

The lymph nodes, both deep and superficial, were enlarged from 0.5 to 1.5 cm. across. Their enlargement was somewhat more pronounced in the single case of lymphatic leukemia. In 8 of the cases the nodes were pale yellowish gray and rather firm. In the cervical regions they formed large masses. In the 4 pigmented cases the nodes were a striking, light lime green color.

The liver in some cases was definitely increased in size and grayish red. In the chloroleukemia cases the site of the leukemic infiltrations about the portal areas was more easily recognized since these foci had an unmistakable green hue. The kidneys were almost constantly enlarged, sometimes to three or four times the normal size. They were occasionally mottled with fresh hemorrhages. The enlargement was chiefly at the expense of cortical tissue, the radial markings of which were quite distinct on cross-section. The green coloration was pronounced in the chloroleukemia cases.

The marrow of both flat and long bones seemed abundant and varied from gray to grayish red. In the pigmented cases the entire skeleton was tinted a light green. This was so striking in the flat bones of the skull that it was faintly visible through the scalp. Occasionally other tissues, such as the salivary glands and lungs, showed the presence of green pigment. If abscesses in the lungs and purulent material elsewhere were present, they too had become pigmented in a similar fashion. All the pigmented areas faded slowly on exposure to air and formalin. The color could be intensified by immersion in hydrogen peroxide. After fixation it could be partly restored in the same manner for several weeks, but later it lost this property entirely. Similar phenomena have been described in studies with human chloroma.

The blood clots in the heart chambers and larger veins were usually pale yellowish gray streaked with red. The blood in the cases of chloroleukemia showed no trace of abnormal pigmentation.

Among the incidental findings at autopsy were collections of pus in the auditory bullae. The incidence of middle ear disease at the time of spontaneous death in this strain is almost 100 per cent, although it is rarely found when the animals are sacrificed at a younger age. In 2 cases the foci of suppuration had extended through the floor of the cranial cavity and given rise to brain abscesses. Abscesses in the lungs and in the uterus were occasionally observed. These incidental findings were of the same nature as those found in the non-leukemic animals. If anything they were less extensive, especially in the younger individuals.

Microscopically the leukemic cells were readily identifiable as myeloid in nature in 11 of the cases. They varied in appearance, many appearing to be quite mature and closely resembling polymorphonuclear neutrophils. The nuclei of these cells were segmented or ring-shaped. Other cells appeared less well developed and had spherical or oval nuclei. Neutrophilic granulations were demonstrated with the Giemsa stain. The vast majority of the cells gave a positive oxidase reaction by the Graham method (para-alpha-naphthol-pyronin). Only a few myeloblasts could be identified. There were, however, many mitotic figures and a moderate number of eosinophils and megakaryocytes. In the single case of lymphatic leukemia the cells were of the small round cell type closely resembling mature lymphocytes and having scanty, non-granular, oxidase-negative cytoplasm.

In the bone marrow the leukemic cells had entirely replaced the normal cellular constituents and the adipose cells. The splenic and lymph node architecture was obliterated by massive accumulations of leukemic cells. The lymphoid follicles and trabeculae could not be seen except in the case of lymphatic leukemia. In the spleen of this animal, huge hyperplastic follicles, often partially coalescent, could be made out, but in places small areas of normal pulp intervened. In the liver, foci of leukemic cells were found both in the periportal spaces and in and along the sinusoids. Where this was most marked the liver cells were compressed and atrophic.

Leukemic infiltrations were frequently found in the renal cortex and peripelvic fat. Here they tended to be patchy. In places the

tubules and glomeruli were widely separated by the cellular accumulations. An incidental renal finding present in every case of leukemia and never observed in any of the non-leukemic animals was the presence of large, well defined colloid droplets in the cytoplasm of practically every convoluted tubule. The nuclei of the lining cells were for the most part intact, but the cytoplasm was filled with droplets which varied in size up to that of an erythrocyte. The droplets were particularly conspicuous in Giemsa preparations. They were found in areas where leukemic cells had not infiltrated and can therefore not be considered as a degeneration due to pressure. The nature of this lesion is obscure, but it very definitely appears to be associated with the leukemic process in these animals.

Leukemic infiltrations were also encountered in other organs, such as the lungs, ovaries, uterus, testes, salivary glands, pancreas and heart. The blood vessel contents were rich in leukemic cells.

One interesting finding was the presence of many large phagocytic cells interspersed among the leukemic cells in all 4 cases of chloroleukemia and in 5 of the non-pigmented myeloid leukemia. These cells were large, pale staining elements with a single, small, round, pale staining nucleus situated in no constant place in the cytoplasm of the cells. The cytoplasm was spongy or even foamy in appearance and included coarse nuclear particles which contained no oxidase granules and only rarely gave a faintly positive Prussian blue reaction for iron. With Sudan III they were shown to be filled with fine droplets of stainable fat.

The cells were particularly striking in the bone marrow, lymph nodes and renal and hepatic infiltrations, and less so in the spleen, although fat stains showed them to be present there as well. They appeared to be somewhat more abundant in the chloromatous rats than in the others. Eichhorst<sup>9</sup> has described similar elements in the bone marrow of a case of chloroma. But their absence in all the other reported cases of human chloroma, as well as their presence in the unpigmented cases of rat leukemia, make it seem unlikely that they have any specific connection with the abnormal pigmentation.

It was impossible to detect any histological difference between the non-pigmented and pigmented cases. The leukemic cells in both had the same appearance. Eosinophils and the more mature forms with polymorphic nuclei were not more abundant in the chloroleukemic animals.



A single attempt was made to transmit the disease experimentally. The spleen from 1 of the cases of chloroleukemia where death took place a few hours previously and which showed only slight post-mortem changes was minced in sterile saline and injected intraperitoneally into 12 stock rats. After 8 months the rats were sacrificed and failed to show any evidence of leukemia. Since we have no information concerning the viability of the cells in the inoculum and since the rats used were not of the same strain as the leukemic rat, the negative result is without much significance.

#### SUMMARY AND CONCLUSIONS

The spontaneous development of myeloid, chloromyeloid and lymphatic leukemia in a strain of inbred albino rats is described. The total incidence of leukemia in this strain was 3.3 per cent. The disease is one of advanced life occurring chiefly in animals over 2 years of age at death and never in those under the age of 18 months. It is somewhat more common in males than in females. No dietary influence on the development of the disease was observed. The pathological changes are described in detail and conform in most respects to those observed in other species, including man.

One incidental finding not described in the leukemia of other species is the presence of colloid droplets in the convoluted tubules of the kidneys. The nature of this nephrosis and its relation to rat leukemia is unexplained.

Lipoid-containing phagocytes are almost constantly associated with the extravascular accumulations of myeloid cells.

No histological differences between the pigmented and non-pigmented forms of the disease were recognized.

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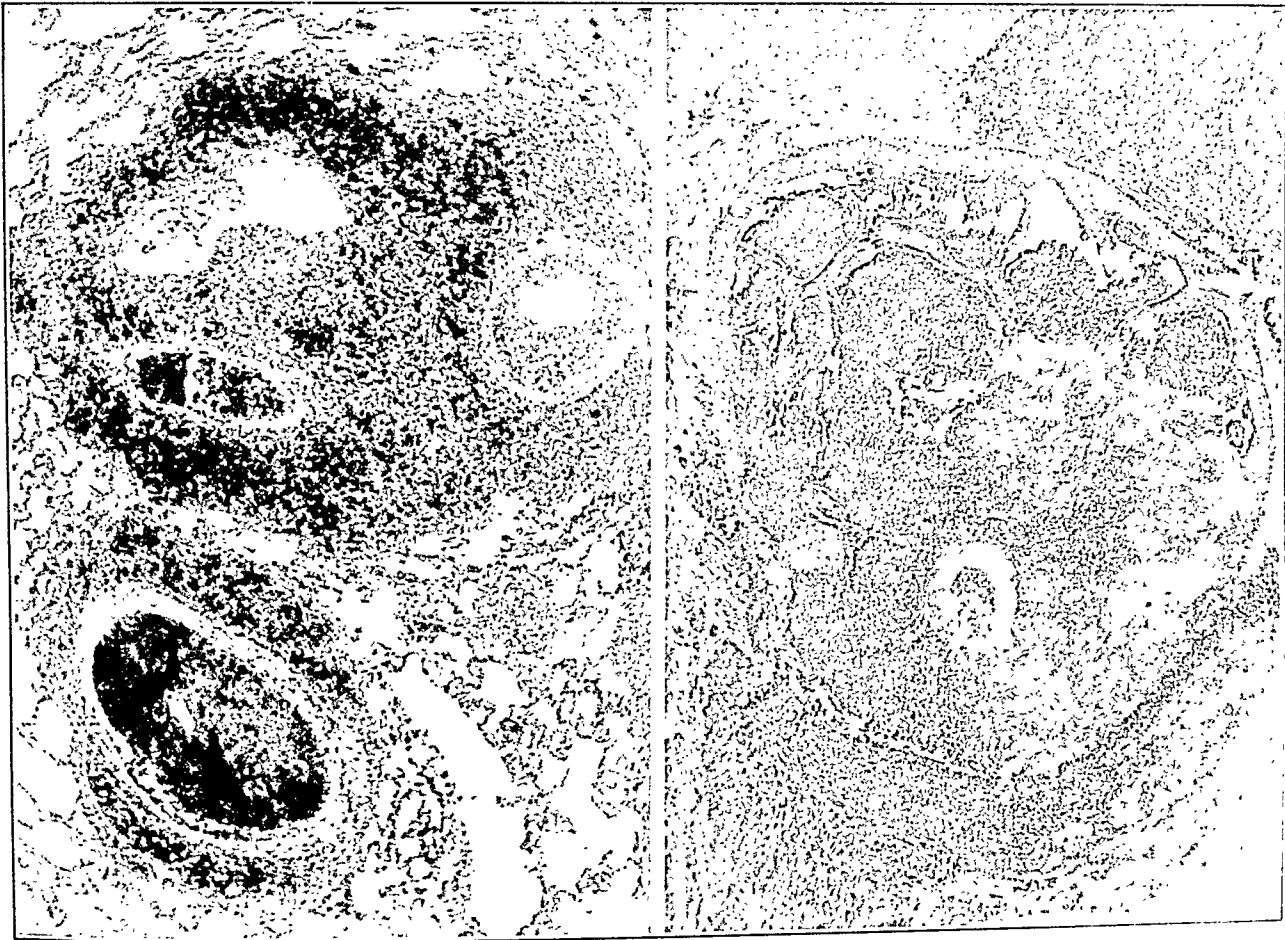
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## DESCRIPTION OF PLATES

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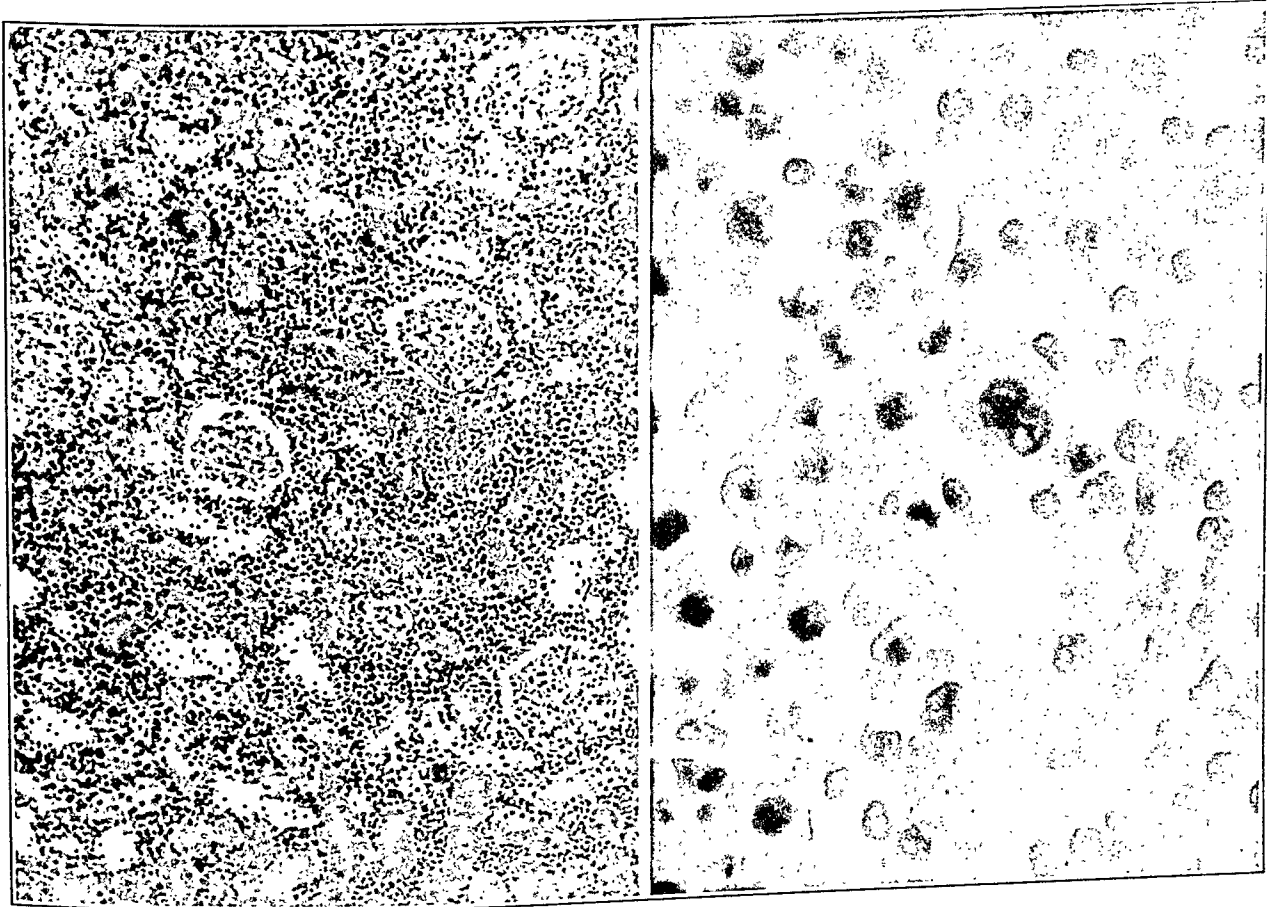
### PLATE 29

- FIG. 1. Myeloid leukemia. Perivascular and peribronchial leukemic infiltrations in lung; lumens of vessels filled with leukemic cells.
- FIG. 2. Myeloid leukemia. Sternum. Hyperplasia of marrow and infiltration of adjacent skeletal muscle.
- FIG. 3. Myeloid leukemia. Infiltration in renal cortex.
- FIG. 4. Myeloid leukemia. Infiltration of hepatic sinusoids.



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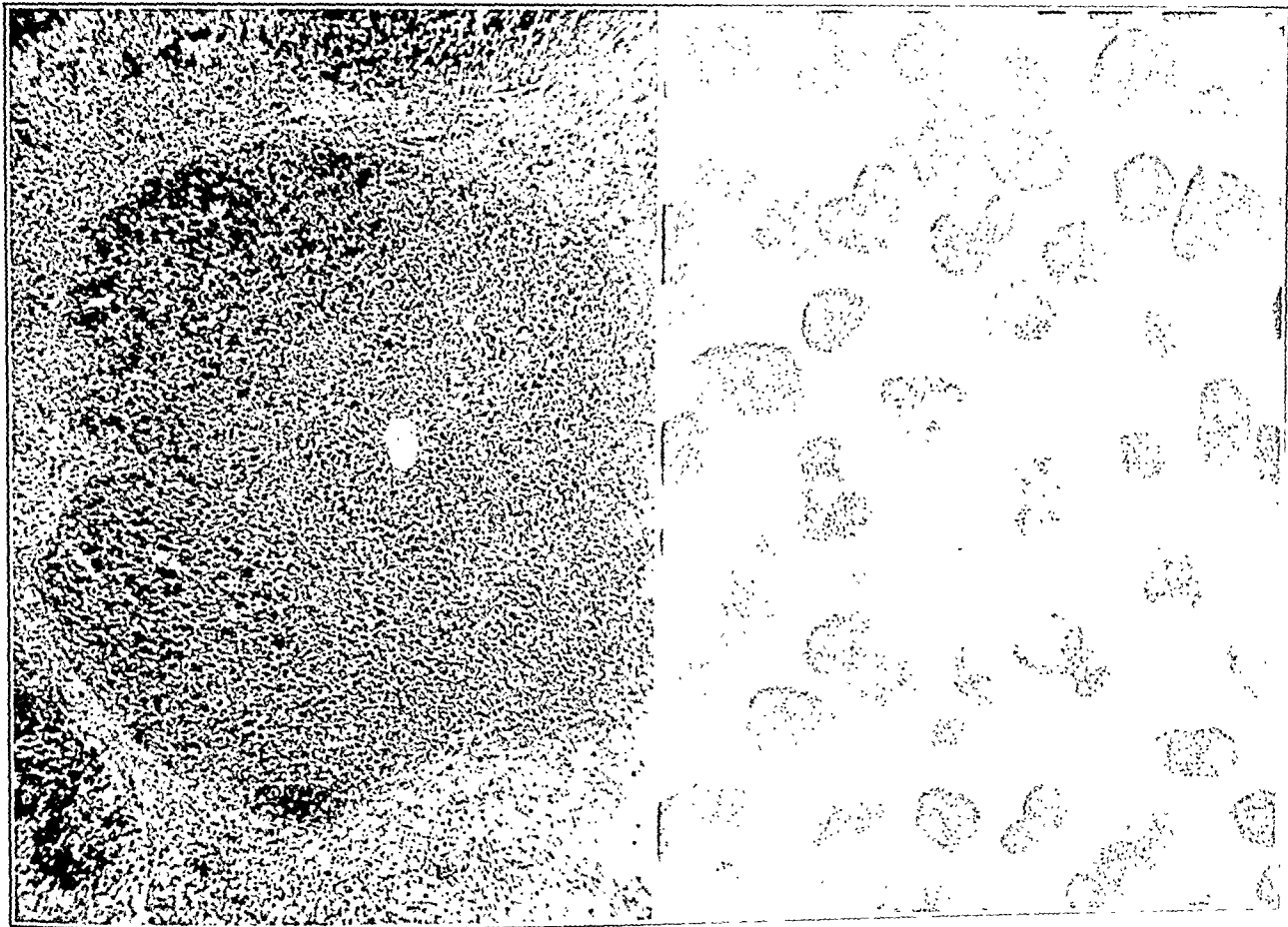
PLATE 30

FIG. 5. Spleen. Lymphatic leukemia. Hyperplasia of malpighian corpuscle of spleen.

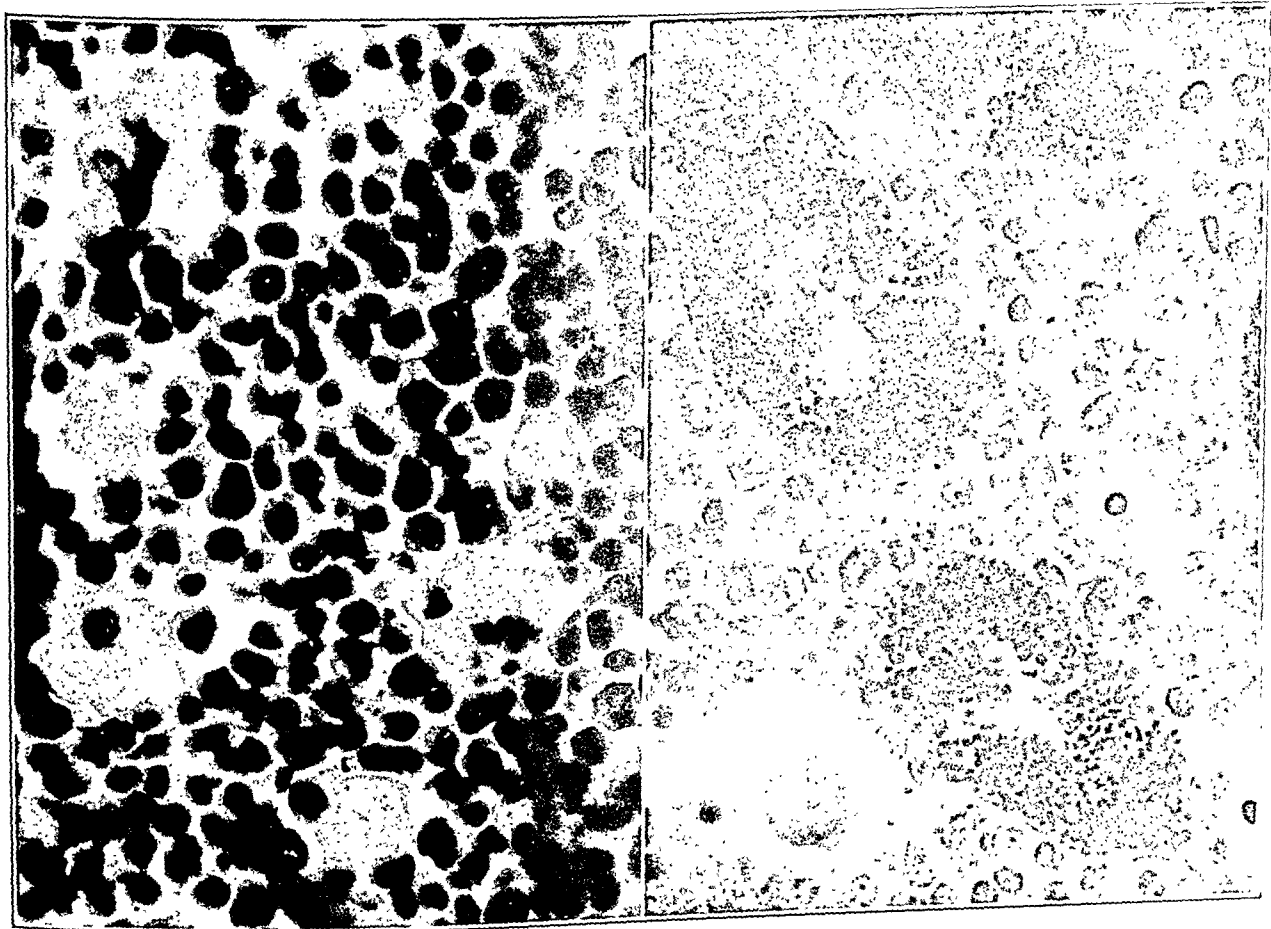
FIG. 6. Myeloid leukemia. Cells in the pulp of a lymph node showing irregular nuclear forms.

FIG. 7. Myeloid leukemia. Lipoid-containing phagocytes in lymph node.

FIG. 8. Myeloid leukemia. Colloid droplets in cytoplasm of convoluted tubular epithelium in kidney.



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## MITRAL STENOSIS WITH INTERAURICULAR INSUFFICIENCY\*

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Mitral stenosis with interauricular insufficiency has been an uncommon finding at autopsy in patients who suffered from cardiac disease during life, and only in the past 20 years have definite clinical and pathological facts been correlated in an effort to define a syndrome with salient diagnostic features. Thus, 1 case is here recorded which falls into this classification, and another presenting certain clinical and roentgenological findings which admit of a similar diagnosis is also reported.

Martineau<sup>1</sup> in 1865 was the first to report this condition in the literature. One year later Peacock<sup>2</sup> reported a similar case, and in the next 7 years 3 other cases were added. Firket<sup>3</sup> in 1880 described in detail the clinical and pathological findings in a case of a large auricular septal defect with associated mitral stenosis in a female, 74 years of age, who had survived eleven full term pregnancies. He hypothesized a favorable rôle of the septal defect in that it relieved the circulatory embarrassment ordinarily found in the pulmonary tree in mitral stenosis, believing that the shunting of the blood from left to right auricle, due to pressure differences, conveyed it to the caval veins, thus acting as a safety valve to the encumbered lesser circulation. Later Lutembacher<sup>4</sup> pointed out that such a transmission was effective only when failure of the right auricle had taken place, the main domain of circulatory changes being confined to the pulmonary veins and artery.

Dr. Maude Abbott<sup>5</sup> in 1915 reported a case of a female, 38 years of age, with a history of cardiac symptoms since 14 years of age following an attack of rheumatic fever. The patient had successfully survived one full term pregnancy. Following several breaks in cardiac compensation she died of circulatory failure. Autopsy revealed an old buttonhole type of mitral stenosis with patent foramen ovale 2 by 1.5 cm. in diameter. Both auricles were greatly enlarged, as were the right ventricle and pulmonary artery. The aorta was hypo-

\* Received for publication October 14, 1935.



plastic. Clinically the patient had shown a faint thrill and presystolic murmur toward the middle of the precordium in the fourth left interspace, and on two occasions a presystolic murmur at the mitral area with an accentuated second sound. Increased pear-shaped dullness of the heart to percussion was noted. Roentgenograms and fluoroscopic examinations were not made and a positive diagnosis of a septal defect with mitral stenosis was not made during life.

The following year Lutembacher<sup>4</sup> reported a case of a female, 60 years of age, who had survived seven full term pregnancies. She had no history of previous rheumatic fever. On admission she presented all the signs of stasis of the systemic veins. Cyanosis was absent. The heart showed a complete arrhythmia but a systolic bruit at the apex preceded by a less intense rumble was determined. In spite of digitalis therapy she shortly succumbed of circulatory failure, exhibiting an intense cyanosis of the lips, cheeks and nose just prior to death. Autopsy revealed an enormous dilatation of the right auricle and ventricle, with considerable muscular hypertrophy. The right ventricle formed the entire anterior aspect of the heart, including its apex. The pulmonary artery was enormously dilated, as were its branches, each one of which was the size of a normal pulmonary artery. The pulmonary orifice was competent, due to the development of the pulmonary valves. Abundant atheromatous plaques were present throughout the pulmonary tree. The aorta was hypoplastic and showed slight atheromatous infiltration. Its valves were undamaged. The mitral valve showed a tight, rigid stenosis, scarcely admitting the tip of the little finger; the tricuspid valve readily admitted four fingers. A large defect in the auricular septum measured 3 by 5 cm.

Drawing conclusions from his case, and others reported in the literature, Lutembacher considered the possibility of the clinical diagnosis and stressed certain anatomical features. Roentgenological examination alone he believed of paramount importance in making the diagnosis, as nothing characteristic could be determined from the cardiac murmurs. Normally in a pure mitral stenosis the outline of the heart is characteristic. The vertical contour of the left border and its pointed appearance bring out at the same time the great development of the left auricle and the relative smallness of the left ventricle. A see-saw-like movement imparted to the heart by the dilatation of this auricle he believed to be more characteristic than

the malformation of the cavity itself. This movement, taking place in a clockwise direction, causes the apex of the left ventricle to indent the diaphragm. The left border of the heart becomes less curved and more nearly parallel with the anterior thoracic wall. At the same time the right border is raised, uncovering the first part of the inferior vena cava. He stressed the fact that when a large auricular septal defect complicates the mitral stenosis the picture is quite different, even disregarding the great dilatation of the pulmonary artery. The greatly dilated right auricle and ventricle give at the same time the appearance of both a transverse heart and a sabot-shaped heart. The movement of its blunted apex is upward and outward in a counterclockwise direction, uncovering all the lower border of the right ventricle normally hidden in the diaphragm. The left contour of the heart takes the form of a broken line. Its superior part obliquely and at the left corresponds to the left ventricle, the right inferior oblique portion to the right ventricle forming its apex.

An interauricular septal defect may be one of several types, depending on the embryological development of the septum primum. In embryos of 6 mm. the septum primum is seen to arise from the dorsal and cranial walls of the atrium, where it grows backward and ventrally to join the endocardial cushions. The primary foramen ovale or interatrial foramen then appears as a narrow aperture between the caudal end of the septum primum and the endocardial cushions. Before these two fuse, a second foramen ovale appears in the dorsal and upper part of the septum primum. The septum secundum appearing in embryos of 9 mm. arises from the ventral and cephalic walls of the atrium just to the right of the septum primum. When the growth of this has caused its dorsal margins to pass beyond the dorsal margin of the secondary foramen ovale, it acts as a flap valve allowing passage of blood from right to left but preventing a reflux. It becomes closed shortly after birth as a result of the raised pressure in the left auricle which approximates the septum primum with the fused left valve of the sinus venosus and septum secundum. Then, when the lower part of the septum primum fails to develop, the defect is termed a persistent ostium primum; when extreme it forms a trilobulate heart. A defect in the upper part of the septum is known as a persistent ostium secundum. However, other factors may produce an insufficiency of the auricular septum, such as perforation of the septum by endocarditic processes, various degrees of

reopening, non-adherent or probe-patent flaps forming the secondary foramen ovale by hypertrophy and dilatation of the auricles due to adherent pericardium, or other causes. Clinically all these cases fall into the acyanotic group. The terminal cyanosis often seen is explained by the mixture of venous and arterial blood by the reverse passage of blood from right to left auricle when compensation in the latter has failed.

Dressler and Rösler,<sup>6</sup> reviewing the subject of mitral stenosis with interauricular insufficiency in 1930, reported a case of a female, 30 years of age, in whom the auricular septal defect was discovered shortly after birth. She was free of cardiac symptoms until the age of 22 years when, following an attack of influenza, she failed rapidly. On examination she presented the stigmata often seen with hypoplasia of the aorta, that is, small stature, slender build and signs of delayed puberty. Slight cyanosis of the cheeks and lips was present. X-ray examination suggested an auricular septal defect because of the large pulmonary artery and underdeveloped aorta. Fluoroscopic studies demonstrated the enlarged, pulsating hilar pulmonary vessels. Electrocardiogram showed an enlarged and split R-wave, being up in lead one, and inverted in lead three. The Q R S time was 0.11. The P R interval 0.17. The T-wave was inverted in all three leads. The most conspicuous findings at autopsy were the marked hypertrophy and enlargement of the right heart, associated with a large pulmonary artery and hypoplastic aorta. The left ventricle appeared as an appendage on the posterior surface of the heart, which had been rotated posteriorly because of the greatly enlarged right ventricle which formed both anterior and left borders of the heart as well as the apex.

McGinn and White<sup>7</sup> analyzed 24 cases of mitral stenosis with interauricular insufficiency (not mentioning the 2nd case of Tylecote<sup>8</sup>) reported in the literature up to 1933, including 1 case of their own. Only 2 of these cases occurred in males.

Dr. B. S. Oppenheimer, at the meeting of the New York Pathological Society in April, 1934, presented 8 cases of gigantism of the pulmonary artery, 1 of which showed the syndrome of a large interauricular defect associated with mitral stenosis, bringing the total number up to 26.

Our case, the report of which follows, is the 3rd one reported to have occurred in a male and brings the number up to 27.

## CASE REPORT

CASE 1. A white male, 67 years of age, was admitted to the medical ward of Saint Luke's Hospital on Dec. 6, 1929, complaining of sore throat, head cold and general malaise. On questioning, he admitted some exertional dyspnea. There was no history of rheumatic fever. Venereal infection was denied.

Examination of the heart showed the left border on percussion outside of the nipple line in the fifth interspace. A thrill and systolic murmur at the apex was transmitted to the axilla. The blood pressure was 104/80. One year later he was readmitted with a diagnosis of auricular fibrillation and acute bronchitis. In 1933 he was twice admitted with cardiac complaints, and blood Wassermanns taken on these occasions were positive. Since his discharge, 5 months prior to his last admission, he constantly suffered from exertional dyspnea, orthopnea and nocturia.

Three days prior to the last admission he began to complain of sore throat, cough and expectoration of frothy, watery sputum. On admission his temperature was 104 F. and he had signs of pneumonia at the base of both lungs. The heart was markedly enlarged both to the right and to the left of the sternum. Systolic and presystolic murmurs were heard at the apex followed by an accentuated first sound. To the left of the sternum in the third and fourth interspace a soft diastolic murmur was heard. The pulmonic second sound was accentuated. The aortic sound was distant. The white blood count was 6800, polymorphonuclears 94 per cent. A portable X-ray was taken of the chest, which confirmed the diagnosis of pneumonia, but the patient was too ill to permit fluoroscopy or further clinical study. Electrocardiograms taken on previous admissions showed normal rhythm, low ST leads one and two, right ventricular preponderance, inverted T in lead three, and notched R in all leads.

The patient succumbed to pneumonia 3 days after admission. An autopsy was performed 7 hours after death and reported as follows.

*Postmortem Examination*

The body was that of a well developed and well nourished male. Icterus was present, most marked over the upper half of the body. Rigor mortis had set in and postmortem lividity was seen over dependent parts. On opening the thorax no free fluid was found in either pleural cavity. The right lung weighed 1275 gm. Firm old adhesions bound the posterolateral surface of the lower lobe to the parietal pleura. The upper lobe on cut section was dry, crepitant and smooth in its upper half, its lower half being irregularly congested. The middle and lower lobes showed a confluent type of bronchopneumonia. The left lung weighed 725 gm. Old adhesions were present over the upper and lateral surface of the lower lobe binding it to the parietal pleura. The lower lobe showed a pneumonic infiltration similar in character to that found in the right lung. The pulmonary artery was markedly dilated in its ramifications throughout the lung tissue. The heart weighed 700 gm. The peri-

cardial sac contained 130 cc. of bile-stained fluid. The transverse diameter of the heart was greatly enlarged, the lateral border of the right auricle extending to the midclavicular line at the level of the fourth rib. The right ventricle formed the greater portion of the anterior surface of the heart and the apex reached the sixth left rib in the anterior axillary line. Large, irregular, white fibrous plaques were present over both the right auricle and the ventricle. The right ventricular wall measured from 15 to 17 mm. in diameter at the base. The left ventricular wall measured 2 cm. in diameter. On opening the mitral valve its leaflets were found thickened, sclerotic and bile-stained. It measured 9 cm. in diameter. The free edge of the anterior flap was more thickened than the posterior valve edge. The chordae tendineae showed some fibrosis and shortening. An oval auricular defect just above the septal flap of the tricuspid valve and below the mouth of the auricular orifice measured 3 cm. in diameter. Its location corresponded to an arrest in the development of the ostium primum or primary interatrial foramen, the secondary foramen ovale being closed and showing no defect. The coronary orifice was dilated, measuring 12 mm. in diameter. The vein was similarly enlarged. The aortic valve measured 6 cm. in diameter. Garland-like, fine verrucae extended down from both sides of the left posterior commissure, 3 mm. below the free edge of the cusps. The valve commissures were fused, but the valve leaflets were quite pliable. The coronary vessels showed a moderate amount of sclerosis and tortuosity. The thoracic aorta measured 6.5 cm. in circumference throughout its extent and was remarkably free from scarring or atheroma. The tricuspid valve measured 14 cm. in diameter, and its flaps were mobile but somewhat sclerotic. The pulmonary valve measured 11.5 cm. and its cusps were sufficient. The pulmonary artery was of a similar circumference to its point of bifurcation. Its wall showed no gross changes.

On microscopic section the aorta showed slight degenerative changes, but no evidence of inflammatory or specific lesion. The pulmonary artery showed slight fraying of the elastic tissue in its media with infiltration of the muscle cells with calcium salts, but no other remarkable changes.

CASE 2. A 2nd case came under our observation presenting X-ray and fluoroscopic signs suggesting mitral stenosis with interauricular insufficiency. The patient was a white male, 41 years of age, who was first admitted on Jan. 7, 1933;

complaining of an attack of dyspnea and palpitation of 4 hours duration. One year previously after a sudden exertion the patient became dyspneic and dizzy and complained of palpitation lasting from 3 to 4 hours. This subsided with rest in bed. Since this attack he suffered from mild attacks of dyspnea and palpitation, always following exertion or excitement. Exacerbation of these symptoms caused him to seek relief at a hospital. He gave no history of rheumatic fever.

Physical examination showed a slightly emaciated, pale white male of asthenic habitus. Paroxysmal tachycardia with ventricular systoles was present. On restoration of normal rhythm, which occurred during examination, the mitral first sound at the apex was partially replaced by a low pitched blow. A faint, light diastolic murmur was heard at the apex. The apical second sound was loud. Systolic blood pressure was 90. The pulmonic second sound was accentuated. Electrocardiogram showed normal rhythm with right ventricular preponderance, high ST in lead one, low ST in leads two and three, low T in lead one, inverted T in lead three, notched R in all leads. X-ray examination showed a moderately enlarged heart with a bulge in the region of the left auricle which overshadowed the aortic region. Considerable thickening of both lung roots was present. Re-examination in the lateral position failed to reveal any bulging of the heart chambers. Fluoroscopic studies were not made at this time. The patient improved with rest and digitalis, and was discharged 3 weeks after admission.

He was in fair health until 4 months prior to his present admission, when he suffered from chest cold, fever, malaise, a non-productive cough and frequent exacerbation of his exertional complaint. The heart was moderately enlarged to the left. The first sound at the apex was faint; the second sound had a rapid reduplication both at the apex and the base. Soft, blowing, systolic murmurs were heard at both the pulmonic and the aortic areas. The pulmonic second sound was increased. Blood pressure was 98/65. Electrocardiogram showed a prominent P wave in lead two but otherwise essentially the same as the previous examination. X-ray retakes showed a mitral configuration of the heart with a greatly enlarged pulmonary artery and a hypoplastic aortic knob. The right auricle was dilated, its contour rounded, particularly at its lower border where it formed an acute angle with the diaphragm. Fluoroscopic examination showed the enlarged shadow of the pulmonary artery to give a strong expansile pulsation.

The patient is at present well and under observation at the cardiac clinic.

### SUMMARY AND CONCLUSIONS

A brief review of several outstanding contributions to the study of mitral stenosis with interauricular insufficiency collected from 26 cases reported in the literature up to the present time is offered and 1 case of our own is added.

The pathogenesis of the septal defect concerns the developmental arrest of the primary or secondary foramen ovale or the reopening of a probe-patent secondary foramen by an acquired hypertrophy and dilatation of the auricles. Perforation by endocarditic processes is rare.

The mitral stenosis is an acquired, not a congenital, lesion, although only 3 cases gave a history of rheumatic fever and one had several attacks of chorea.

The syndrome is rare in males, only 3 cases including our own being reported.

The stigmata associated with hypoplasia of the aorta, that is slender build, small stature and delayed puberty, may or may not be present. The symptoms most frequently complained of are exertional dyspnea, palpitation and easy fatiguability. Cyanosis is absent, or present only as a terminal event. Club fingers were reported in only 1 case. Auscultatory signs are variable and may be absent. An apical systolic murmur is most frequently heard, being present in 16 cases. It was accompanied by a diastolic murmur maximal at the apex in 9 cases. An apical thrill was present in 5 cases. The pulmonary second sound is usually accentuated. Three cases had electrocardiograms which showed a right ventricular preponderance and notched R-waves in all leads. P-waves showed no distinct abnormalities. All the hearts were enlarged, due mainly to dilatation and hypertrophy of the right auricle and ventricle. The left auricle was enlarged, but did not attain the size of the right auricle. The left ventricle was usually small and rotated posteriorly, unless there was a complicating aortic valve lesion. Dilatation of the pulmonary artery with a small aorta was present in 16 cases.

Roentgen examination demonstrates these anatomical points and is of paramount importance in making the diagnosis. Fluoroscopy shows the right ventricle to form the greater part of the anterior surface of the heart and its apex, imparting an upward and outward movement to the latter. There is an expansile pulsation of the pulmonary vessels at the hilum.

The prognosis simulates somewhat that of mitral disease. In 13 of the 27 cases death occurred at 30 years of age or under, the average age at death being about 35 years. Females have been noted to survive several pregnancies with this condition.

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## DESCRIPTION OF PLATES

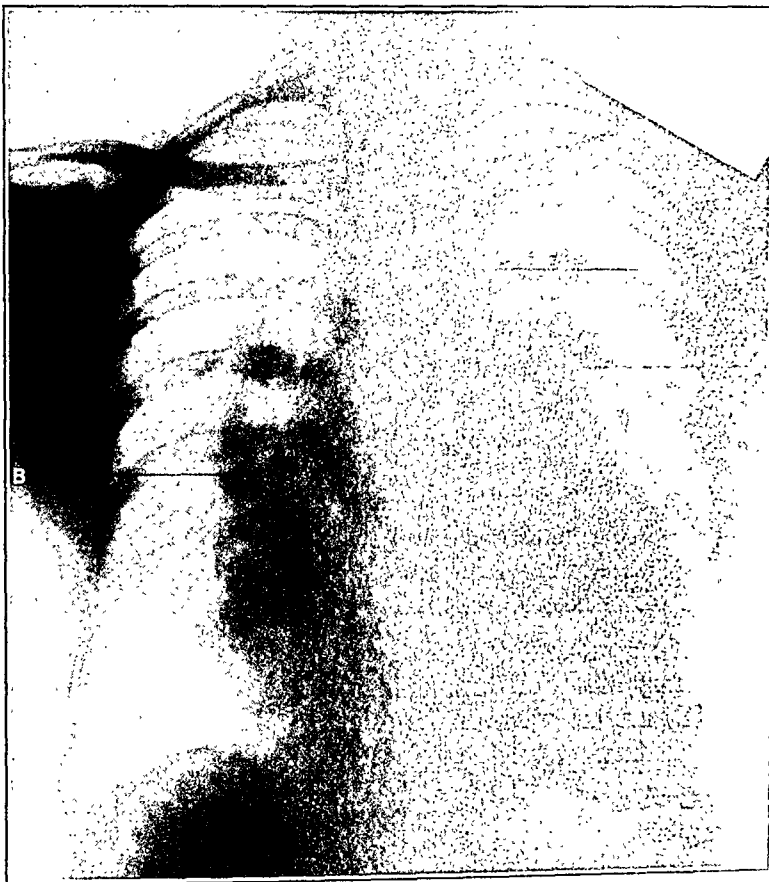
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### PLATE 31

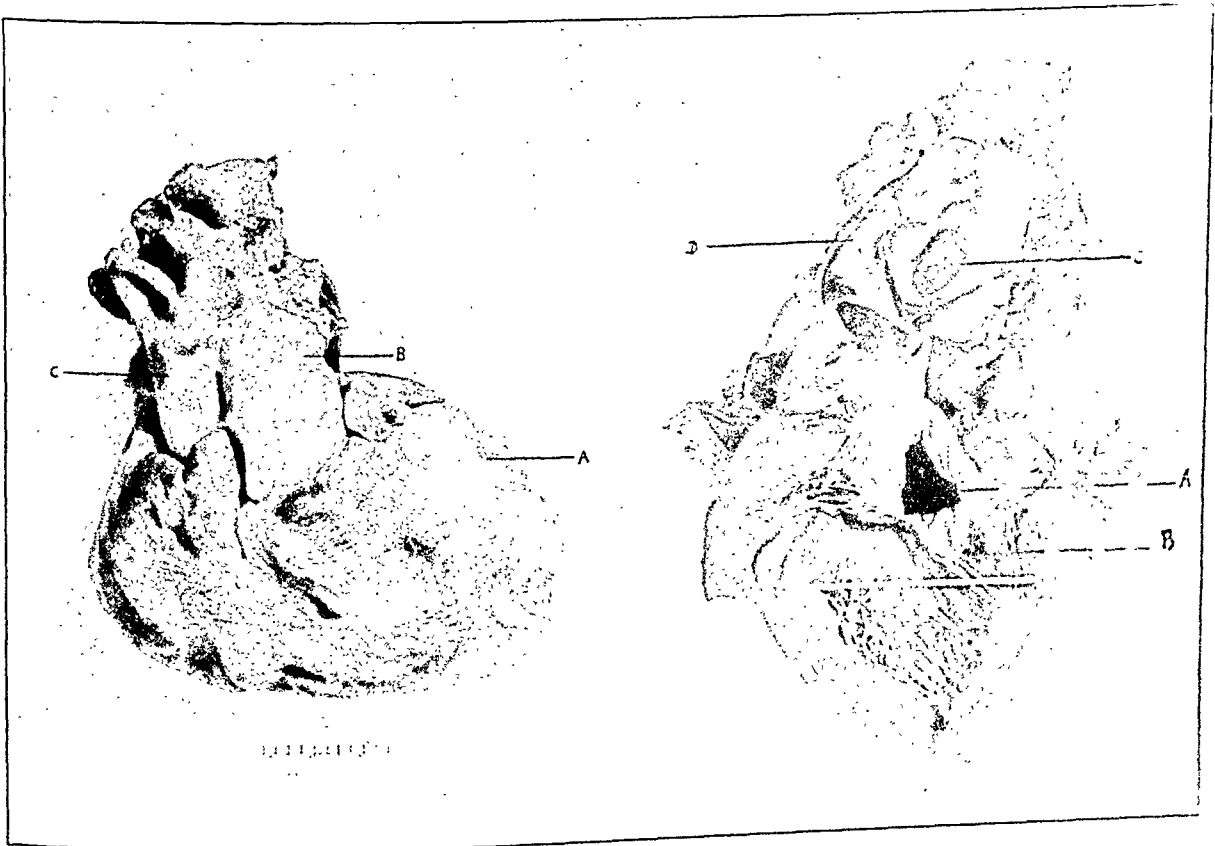
FIG. 1. Chest X-ray plate in anteroposterior diameter. Large transverse diameter of heart, small aortic knob (C). Large pulmonary conus (A) and prominent right hilar shadow (B) — pulsating pulmonary artery. Prominent contour of rotated, dilated right auricle.

FIG. 2. Anterior view showing position of heart as it appeared in the thoracic cavity. The right-sided hypertrophy rotating the left ventricle (A) posteriorly and the disproportion between the pulmonary artery (B) and aorta (C) are apparent.

FIG. 3. Heart opened from left side. Large auricular septal defect (A) and thickened, contracted mitral cusps (B) visible. Enlarged branch of pulmonary artery (C) and its relations to the aorta (D) can be seen.



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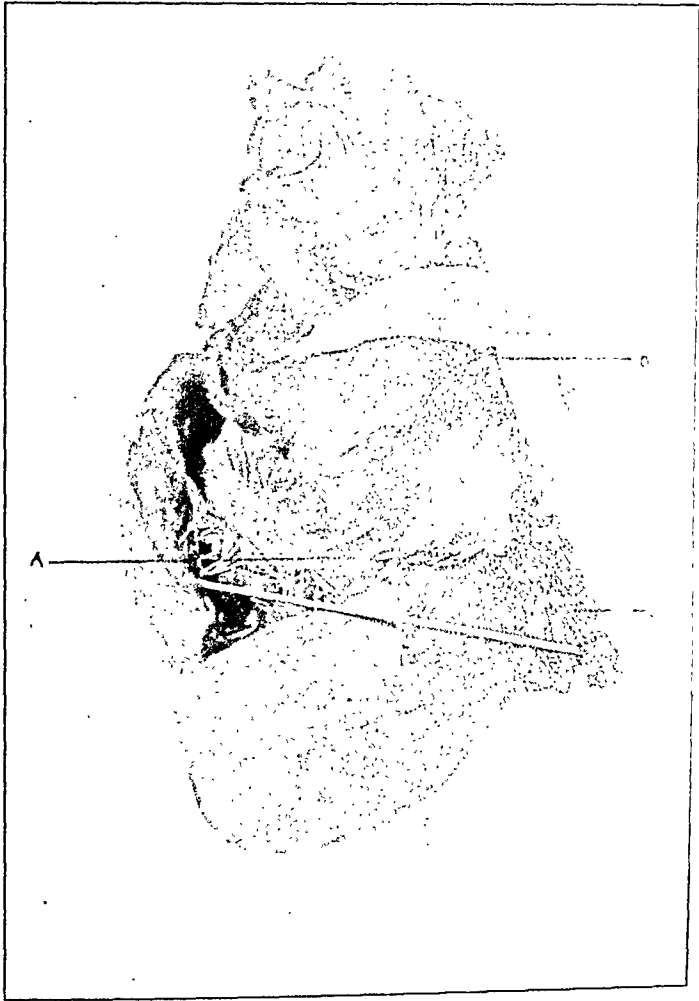
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PLATE 32

FIG. 4. Right side of heart. Auricular defect (A) just above membranous portion of interventricular septum. Right auricle (B) greatly dilated and hypertrophied. Right ventricular wall (C) hypertrophied.

FIG. 5. Chest X-ray plate in anteroposterior diameter. Greatly enlarged pulmonary artery (A) and inconspicuous aortic knob (B). Enlarged hilar shadows (C) are branches of the dilated pulmonary artery which are seen to pulsate on fluoroscopic examination.

FIG. 6. Lateral view of same patient showing relative proportion of enlarged pulmonary artery (A) and small aorta (B).



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# THE BRAINS OF INFANTS AND CHILDREN IN RELATION TO POSTMORTEM TIME, TOXICITY AND CONVULSIVE STATE \*

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Aside from the well defined nervous diseases of childhood, neuropathology has occupied itself chiefly with the study of adult brains. One of the first exceptions was the examination made by Brown and Symmers<sup>1</sup> of the brains in 8 children suffering from an unusual syndrome of convulsions, diarrhea and fever. Since they found an edema of the ganglion cells and of the endothelium of the blood vessels, they concluded that the cases were serous encephalitis. Following this, Grinker and Stone<sup>2</sup> described the neuropathological findings in 10 children suffering from acute infections such as bronchopneumonia, septicemia and scarlet fever, complicated by various nervous manifestations. Their chief observations were swelling and chromatolysis of the ganglion cells, neuronophagia, and an increase in the cytoplasmic glia and endothelium. They believed that these changes were evidences of toxic encephalitis. Under a similar title Low<sup>3</sup> presented the cellular reactions in the brains of 5 children with endocarditis, colitis and bronchopneumonia associated with convulsions, coma and rigidity. He divided his cases into two groups: (1) those in which the ganglion cells were liquefied, the peracute stage; and (2) the acute stage, where vacuoles were present in the ganglion cells. Regressive glial changes accompanied the former and progressive ones the latter.

## MATERIAL AND RESULTS

The present examination of 34 children's brains has been undertaken to determine what may be considered normal and what pathological alterations. The ages of the children were from 12 days to 4 years and the diseases the usual ones of childhood. From most of the brains blocks had been taken and fixed in alcohol. Other brains were fixed *in toto* in formalin and blocks removed after 2 weeks. In some cases the brains remained longer in formalin, but not more

\* Received for publication October 16, 1935.

than a month or 6 weeks, since too long fixation causes cell shrinkage and difficulty in staining. In all the formalin-fixed brains the blocks were washed for 2 days in running water before dehydration. Also, the cases were limited to those autopsied within 15 hours of death, as a longer period of time produces an architectonical distortion from a softened cortex, which is further complicated by the enlarged spaces about the blood vessels. The cytoplasm of the upper cortical ganglion cells is, in general, completely destroyed, while it varies in amount in the lower cells. All the cells are thus apparently decreased in size. The cytoplasm may take a deep stain with pseudo-Nissl substance sharply contrasting with the almost invisible glia, or the glial nuclei may appear dark, small, beaded and not unlike those of the ganglion cells with a poorly staining nucleolus. In other cases the scanty cytoplasm and nuclei may seem fairly well stained, but the nuclei are so much alike that the different types of cells are not easily distinguished except for the larger ganglion cells. In the basal ganglia the cells are shrunken and the cytoplasm may be pale or deeply stained with a usually dull, polymorphous nucleus. Within the confines thus established the similarities and variations in the appearance of the ganglion cells allowed the formation of three groups.

GROUP 1. By the thionin blue stain, the cortical ganglion cells are poorly and irregularly stained, varying from pale pink or blue to dark blue. In the formalin-fixed material the cytoplasm and nuclei are darker but less distinct in the bluish tinged nerve tissue than in the alcohol-fixed material. The barely discernible outline of most of the cells appears to give them a normal size, but the decreased cytoplasm suggests a poorly developed cell. Many ganglion cells in the upper cortical layers have no cytoplasm. That of others is thin and granular, often limited to small particles at the border of the cell or to a narrow strip around the fairly large nucleus. The latter generally stain like the cytoplasm, but some are opaque and violet-hued. Narrow ganglion cells with a deeply stained cytoplasm and nucleus are also numerous. Figures 1 and 2 present a fairly typical picture of the disintegrated and poorly stained cytoplasm in the upper cortical cells. In the lower cortical layers the entire cytoplasm of the larger cells is frequently present. The ganglion cells of the cerebellum, the basal ganglia, the pons and the medulla oblongata are moderately dark blue or pink with a fair abundance

of cytoplasm and some Nissl substance in the alcohol-fixed material but little or none in the formalin-fixed. Central chromatolysis is found in a few scattered cells of the basal ganglia. The cytoplasm of the glia is seldom visible. In the oligodendroglia the nuclei are small and deep blue, while those of the astrocytes are large and vary from light blue to dark, the latter in cases of longest post-mortem time and fixation in formalin. Few microglia are stained, the nuclei taking a paler blue stain than the oligodendroglia. The vessels are somewhat pulled away from the nervous tissue, the space being more pronounced when the postmortem time is prolonged. Perivascular cells and glial reaction are present in the cases of meningitis, together with demyelination in occasional foci of acute inflammation. Microglial arborization is distributed along the capillaries in the molecular layer of the cerebellum in the cases of tubercular and acute meningitis.

*Cases of this Group: (Division A.)* Ages: 12, 14, 15, 17, 24 and 35 days. Autopsy time: 12, 6, 6, 2, 10 and 12 hours postmortem. Cases: cerebral hemorrhages and softening in a convulsive state of 9 days duration; dermatitis; hydrocephalus with small hemorrhages and softening in the white substance together with many ischemic cells in the lower cortex; septicemia; Mongolian idiocy with malformation of the heart; and intestinal toxemia with delayed cerebral development.

*(Division B.)* Age: 2 months. Autopsy time: 8, 12 and 12 hours postmortem. Cases: intestinal toxemia; cellulitis; and bronchopneumonia.

*(Division C.)* Ages: 7, 8, 10, 10½, 12, 18, 30 and 36 months. Autopsy time: first case 10 hours, the others 12 hours postmortem. Cases: pyelonephritis; tubercular meningitis; acute meningitis (2); bronchopneumonia; hemorrhagic purpura of 2 days duration; oxycephaly, with malignant tumor of one kidney and bronchopneumonia; and cellulitis. Convulsions were present in the cases of meningitis and hemorrhagic purpura. The ninth case was a child of 6 months who had suffered for several weeks with diarrhea and secondary anemia. A second blood transfusion, given at a week's interval from the first, was followed by convulsions and death in an hour. Autopsy time: 15 hours postmortem. The ganglion cells are like those of the group except for the many ischemic ones, which are found either isolated or in small bleached foci. The accompanying



glia are unstained or pale. Aside from these areas and apparently independent of them, the macroglia of both the cortex and the white substance show a cytoplasmic increase and the microglia are increased in numbers, their bodies frequently swollen and the processes lost. This reaction of the glia, shown in Figure 3, is most pronounced in the lower cortical layers. A perivascular demyelination seems to have occurred in the white substance, although the usual fat deposit in the perivascular spaces is not increased.

GROUP 2. The outline of the cortical ganglion cells is sharper than in the previous group and more distinct in the alcohol-fixed material than in the formalin-fixed. A slight pink or bluish tinge is given the nerve tissue. Many cells of the upper cortical layers and most of those of the lower are filled with a faintly pink or dark violet granular cytoplasm in which Nissl substance is not visible. Other ganglion cells have very little cytoplasm and a rather punched-out appearance, as in Group 1. A few cells are small and dark. The nuclei of the ganglion cells are large and stain like the cytoplasm, occasionally lighter or darker. In other parts of the brain the cytoplasm fills the cells and is deep pink or blue, showing Nissl substance in most cells. The nuclei of the glia are of fair size and less deeply stained than in the first group, but the cytoplasm is shadowy. Most of the vessels are not detached from the nervous tissue. In the case of meningitis the same hyperplastic glial changes are present as have been described in the first cases, but the microglial cytoplasm is better stained; microglial arborization is, also, a pronounced feature in the molecular layer of the cerebellum.

*Cases of this Group: (Division A.)* Ages: 2 and  $3\frac{1}{2}$  months. Autopsy time:  $1\frac{1}{2}$  and  $3\frac{1}{2}$  hours postmortem. The first case was one of amyotonia congenita beginning in the 2nd week of life. Later the child had to be tube fed and died of bronchopneumonia. Aside from the description of the cells already given, characteristic central chromatolysis of the ganglion cells is found in the nuclei of the pons, the medulla oblongata and of the basal ganglia. Many of these cells are undergoing neuronophagia by microglia.

The second case was a previously healthy child of  $3\frac{1}{2}$  months in whom an attack of vomiting preceded the onset of successive convulsions and cyanosis. The temperature rose to  $104^{\circ}$  F., where it remained until death on the 2nd day. The only pathological changes noted at autopsy were otitis media and congestion of the

lungs. Ischemic ganglion cells are prominent, though isolated, in the cortex. Developmental retardations are also observed. The vessels are dilated with a few agonal perivascular red cells. In the molecular layer of the cerebellum are extensive foci of microglial arborization.

(*Division B.*) Ages:  $4\frac{1}{2}$  months to 2 years. Autopsy time:  $5\frac{1}{2}$  to 8 hours postmortem. The clinical histories were those of intestinal toxemia; malnutrition with bronchopneumonia; acute meningitis with cortical ischemic cells; and whooping cough of 2 months duration, ending in a temperature of  $105^{\circ}$  F. for 5 days and bronchopneumonia. The following cases are unusual. One was a child of 11 months with whooping cough of several weeks duration. Frequent convulsions and a temperature of  $103^{\circ}$ – $105^{\circ}$  F. developed in the 2 days previous to death. Necrobiosis or bleaching of all but a few cells occurred in both cornua ammonis, one of which is seen in Figure 4. Extensive bleaching of the cortex and the marginal white substance may be observed adjacent to the cornu ammonis in the same figure. Large and small areas throughout the cortex are similarly affected. The ganglion cells in these areas are chiefly ischemic ones, triangular shadow forms with homogeneous, pyknotic, triangular nuclei. Other cells are pale with normal appearing nuclei. The glia are few, showing either a pale or a pyknotic nucleus. Fairly well stained ganglion cells of this group are still present in the vicinity of the larger vessels and constitute a characteristic finding of the necrobiotic regions. In the apparently unaffected cortex surrounding the bleached areas are numerous ganglion cells of irregular shape. Their dark distorted nuclei in more or less pale cytoplasm proclaim them ischemic cells, less completely destroyed than the shadow forms. Occasionally, nuclear bits are present in and outside of the cytoplasm. The neighboring glia have also been affected, being either absent or staining poorly. Agonal hemorrhages are seen in the cortex and the vessels are dilated.

The sixth case, a child of  $8\frac{1}{2}$  months, had a cough (whooping cough?) for 1 week. Sudden convulsions and cyanosis occurred with death in 24 hours. The Sommer sector of one cornu ammonis is entirely bleached. Microglial arborization is marked in the molecular layer of the cerebellum. An occasional ischemic cell is found in the cortex.

The seventh case was a previously healthy child of 8 months who became ill with a rash followed on the 2nd day by a convulsion of

1½ hours duration. The temperature rose to 104° F. On admission to the hospital the convulsions became continuous. The disease terminated fatally on the 4th day with a temperature of 107° F. The blood vessels of the cortex and white substance are markedly dilated. Numerous cortical ganglion cells and those of the Sommer sector of one cornu ammonis are ischemic. The entire cornu ammonis of the opposite side is necrobiotic. In Figure 5 are a few ischemic ganglion cells of this destroyed area. Widely distributed microglial arborization is found in the molecular layer of the cerebellum. Ischemic ganglion cells and pallor of the nerve tissue are present in the thalamus and head of the nucleus caudatus. The cells are less triangular than those of the cornu ammonis but the nuclei are similar.

GROUP 3. Autopsy time: 1 to 4 hours. Ages: 7 months to 4 years. The majority of the cortical ganglion cells of this group are filled with a smooth or finely granular cytoplasm staining a distinct blue in formalin-fixed material or a deep bluish pink in alcohol-fixed material. The cells are clearly dissociated from the surrounding glia and unstained nervous tissue. A small amount of Nissl substance is found in a few lower cortical cells in the case of 7 months, while a larger amount is present in more of the same cells in the older cases. All the nuclei are large and stain somewhat paler than the cytoplasm, though distinct. Figures 6 and 7 illustrate the better preservation and staining of the ganglion cells in this group than in the first one. The cytoplasm of the oligodendroglia and microglia is either pale pink or bluish in color; that of the macrocytes is colorless or pale pink. The nuclei of the latter are very light blue; those of the oligodendroglia are much smaller and moderately dark blue in color, while the nuclei of the microglia vary in size, shape and depth of bluish stain. The vessels are not separated from the nervous tissue. The cells of the medulla oblongata, the cerebellum, the pons and of the basal ganglia are filled with distinctly stained cytoplasm and Nissl substance.

*Cases of this Group:* Acute peritonitis and otitis media of 5 days duration with a temperature of 105° F. in a child of 20 months; second degree burns with severe shock and death in 24 hours, age 4 years; whooping cough of 5 weeks duration with fairly numerous convulsions during the last week, bronchopneumonia, and a terminal temperature of 109° F., age 7 months. The last 2 cases have

small agonal hemorrhages in the cortex. The fourth case was one of lead poisoning of 5 months duration in a child of 2 years. Frequent convulsions, rigid neck and uncertain gait were the chief symptoms in the first few months. Under treatment the child improved, but suddenly died in coma. The meninges are slightly thickened. The astrocytes in the gray and white substance are increased in size and number, as are also the microglia. The oligodendroglia show little change in size or frequency. In many areas the spaces between the cortical ganglion cells appear to be widened and partially filled with glia. Small agonal hemorrhages are found here and there in the cortex. Many Purkinje cells are missing in the cerebellum, while numerous foci of microglial arborization with dark blue-staining deposits are present in the molecular layer. The ganglion cells of the Sommer sector and in the end leaf of one cornu ammonis are entirely replaced by glia.

## EXPERIMENTAL

### *Time Postmortem*

Three 3 months old healthy kittens were killed with ether and autopsied under various conditions. One was immediately autopsied; one-half the brain was placed in 95 per cent alcohol, the other half in formalin. The second kitten was left at room temperature for 12 hours and the third was kept in the icebox for 12 hours. Both were then autopsied and the brains fixed as in the first kitten. The ganglion cells of the first kitten are sharply stained against a clear background, with Nissl substance in the larger cells. The glial cytoplasm is faintly stained and the nuclei of good size and color. Slight shrinkage of the ganglion cell cytoplasm occurs in the formalin-fixed material. In the second and third kittens the cytoplasm in many ganglion cells appears pale, thin and punched out, particularly in the frontal and occipital poles. The nuclei vary in shape and tend towards a darker tint than the cytoplasm. Many cells are narrow with deeply stained cytoplasm and nuclei. Figures 8 and 9 show the normal cell and its postmortem dissolution. Other findings in the second and third kittens are a bluish stained nervous tissue and a widened space between it and the blood vessels. The glial nuclei are small and deeply stained, more marked in the formalin-fixed material and in the kitten left at room temperature. The cells of

the basal ganglia, the pons and cerebellum are fairly well stained with Nissl substance in the larger cells. In the cord the anterior horn cells contain vacuoles. Perivascular hemorrhages are present in the cortex and white substance.

### *Carbon Tetrachloride Poisoning*

Three adult healthy rabbits were exposed to the fumes of carbon tetrachloride. One died from overdosage at the first administration. The other two were placed in an airtight box with 100 cc. of the fluid. After a few minutes a twitching of the nose and shaking of the feet occurred. These signs of irritation were followed at varying intervals by gasping for air, general or localized convulsions and, lastly, by complete anesthesia. Twice daily the rabbits remained in the box from 20 to 50 minutes, depending upon the rapidity of their reaction. One rabbit, after 6 days of the experiment, was not removed until after it had been exposed for 1 hour to the drug, since no response had been obtained. This animal died in the night from convulsions. The third rabbit was killed with ether after 10 days, when it showed some loss of weight and was less easy to arouse from the tetrachloride. The cortical cells are well stained in the first and third rabbits. The glia cells do not appear to be increased in number or size. The second rabbit, autopsied more than 12 hours after death, shows deeply stained, small nuclei in all cells and marked cytoplasmic loss in the ganglion cells in all parts of the brain. The nervous tissue is also bluish and wide spaces are present about the vessels. The latter contain many postmortem bacteria. An increase in the glia is not observed.

### DISCUSSION

The loss of cytoplasm in the ganglion cells of the first group and in normal kittens autopsied after 12 hours has been called liquefaction by Low.<sup>3</sup> He does not consider this a postmortem change but a toxic one, since the oligodendroglia he found within the border of the liquefied cells appear to denote neuronophagia. Oligodendroglia are frequently found within well preserved ganglion cells, and like those in the postmortem autolyzed cell of the normal kitten in Figure 9 seem to indicate an overlapping of cells rather than neuronophagia. If true neuronophagia were taking place, then numerous microglia would be found in and about the cells. Low further doubts that a

selective postmortem influence could produce liquefied cells only in the cortex, since he did not find them in the basal ganglia. As liquefaction, or autolysis (the preferred designation) did not appear in the cells of the basal ganglia of Group 1 or of the late autopsied kittens, it is suggested that postmortem changes may be hastened by bacteria which should find the large vascular bed of the cortex a more fertile ground than the basal ganglia. Also, when the time between death and autopsy is prolonged, the same autolysis occurs in the basal ganglia cells. Chromatolysis of the cells was stressed by Grinker and Stone<sup>2</sup> as characteristic of toxic reaction. Some chromatolysis occurred in the brains of shortest postmortem time, but it was chiefly demonstrable in the cortical ganglion cells of long postmortem time. As the Nissl substance can be stained at the 7th day in the basal ganglia and in some cells of the cornu ammonis, but not until the 7th month in the cortical cells of this series, its absence appears less a developmental process than a susceptibility to postmortem influences. The central chromatolysis in the basal ganglia cells was so frequent that it is not considered of pathological importance.

It may seem that the dissolution of the cytoplasm in the cortical cells in the first group is evidence of a toxic destruction. However, the diversity of the diseases in this group offers no common factor as the source of toxicity. While the ninth case offers some difficulty, because it is clearly one of toxicity, no apparent relation exists between it and the other cases of the same group. On the other hand, the cases of meningitis, hemorrhagic purpura and intestinal toxemia do not appear distinct from similar cases in the other two groups but they are separated by greatly dissimilar cells. It is obvious that the ganglion cells in different brains may suffer individual reactions to toxicity, but there must be some equality between the appearance of the ganglion cell and the clinical history, before the density of the cytoplasm and stain can be regarded as proof of toxicity. Scherer<sup>4</sup> does not consider the ganglion cell particularly sensitive, since normal ones may be found in vascular lesions, in areas of multiple sclerosis, in gliomas, and in parts affected by Wilson's disease. Certainly the toxicity of the cases of lead poisoning and second degree burns cannot be questioned, and yet the ganglion cells show little of the cytoplasmic disintegration and pallor of the first group. Nor is the difference in the appearance of the cells in the cases of whooping

cough in the second and third group explainable on the basis of toxicity, as the symptoms appear to be of like severity. Only the autopsy time seems to unite and divide the cases. The loss of cytoplasm, the poor and irregular staining of the cells, the large perivascular spaces, hyperchromatic glial nuclei and the tinged nerve tissue found in the late autopsied kittens are undoubtedly postmortem changes and similar to the findings in the first group, a similarity which supports the formation of the groups according to the postmortem time.

The exceptions to the groups are the youngest cases, as they are all in Group 1 regardless of the autopsy time. The earliest recognizable influence of the autopsy time on the cortical ganglion cells occurs at 2 months in this series, since the case of amyotonia congenita, autopsied at  $1\frac{1}{2}$  hours, is placed in Group 2, while other cases of the same age, but longer postmortem time, are in Group 1. This disease affected so many of the cells in the cord, the thalamus and the nuclei of the pons and medulla oblongata, that the cortical ganglion cells might be expected to resemble those of Group 1, if this group really represented pathologically changed cells. On the other hand, the cortical cells are not as well stained as in older cases of the same or longer postmortem time. But since it is not possible to claim a more pronounced toxicity for the case of amyotonia congenita than for those in Group 3, or to recognize its similarity to all those in Group 2, it may be concluded that the difficulty in obtaining well stained cortical cells in the youngest infants tends to confirm the contention of Spielmeyer<sup>5</sup> that the brains of infants undergo rapid postmortem dissolution. The explanation for this may be found in the high water content of the brain at birth and in the chemical changes which occur during the first 6 months of life.<sup>6</sup> The determining factor of age in regard to postmortem dissolution ends at 7 months in this series, as the cells in the case of whooping cough with convulsions and high fever are similar to those of Group 3.

The growth of the microglia and of the oligodendroglia, the increased size of the macroglia, together with the demyelination in the foci of inflammatory cells, are the expected reactions to infection in the cases of meningitis. The hyperplasia of the macroglia and of the microglia in the cases of lead poisoning and death following a second blood transfusion not only occurred independently of the ganglion cells (1 case is in Group 1, the other is in Group 3) but was so dis-

tinct and so pronounced in both the gray and white substances as to indicate that the important elements in toxicity are the macroglia and the microglia. Although the oligodendroglia must undergo some change in toxicity, their rôle is not easily demonstrated among the flamboyant forms of the astrocytes and microglia. The absence of glial reaction in the other cases of allergy suggests that the blood transfusion case was one of longer toxicity than the symptoms indicate, perhaps beginning at the first transfusion. Glial changes were not present in the rabbits subjected to carbon tetrachloride; this may mean either that the drug does not produce the type of irritation needed to affect them, or that the experiments were terminated before a glial response could take place. The glial findings of Biancalani<sup>7</sup> could not, therefore, be confirmed, although he obtained the same nervous symptoms during similar brief exposures to carbon tetrachloride. The "liquefied" ganglion cells in his rabbits are identical replicas of the postmortem autolyzed cells in the kittens and rabbit of this study.

It is evident that the most important lesions in this series are the bleached areas of the cornu ammonis, the scattered ischemic ganglion and glia cells, the microglial arborization in the molecular layer of the cerebellum, the ischemic cells of the thalamus and nucleus caudatus and the bleached areas of the cortex, all related to the convulsive state. Since Purkinje cells were not missing in these cases and the disturbed cerebral areas were free of the mesodermal activity which shortly follows injury,<sup>8</sup> death must have succeeded the cerebellar and cerebral changes by only a brief interval. In the case of lead poisoning, the glial scar of one cornu ammonis, the glial lawns of the cortex, and the loss of Purkinje cells are evidences of old injury produced by the convulsions which occurred several months previous to death. As epilepsy has been shown to cause bleaching of the cornu ammonis and microglial arborization of the cerebellar molecular layer, Spielmeyer<sup>9</sup> believed that these areas, because of their poor blood supply, are especially vulnerable to the vascular contractions which sometimes occur in convulsions. The closely interwoven vascular net of the cortex demanded another explanation of its bleaching, and for this, Spielmeyer<sup>10</sup> proposed the hypothesis of localized vascular spasms. Recent experiments<sup>11, 12, 13</sup> in regard to the vascular status in convulsions have neither proved nor disproved Spielmeyer's theory of necrobiosis. The preservation of the ganglion cells



around the larger vessels in the bleached areas, as can be seen in Figure 4, seems to indicate that the smaller vessels and capillaries are the worse sufferers. The widespread ischemic cells in the cortex and in the basal ganglia, the latter a less frequently noted change, suggest not so much a localized spasm as a nervous disturbance of the entire capillary bed. The dilated vessels may mean either a rebound from contraction or a long dilatation which could produce the same lesions as a contraction. They may also have caused the perivascular hemorrhages of the cortex either before or during the time of death.

#### SUMMARY AND CONCLUSIONS

A study of 34 infants' and young children's brains from cases of acute infections, second degree burns, allergy and lead poisoning did not show changes in the ganglion cells that could be attributed to toxicity. The loss of cytoplasm and the density of its stain, the tingeing of the nerve tissue, the hyperchromatosis of the nuclei and the perivascular spaces varied somewhat with age and the fixative, but chiefly with the postmortem time. The indications of toxic injury were found rather in the reactions of the microglia and the macroglia.

The most severe and frequent lesions in the brains were those produced by the vascular disturbances of the convulsive state which accompany many diseases of infancy and young childhood.

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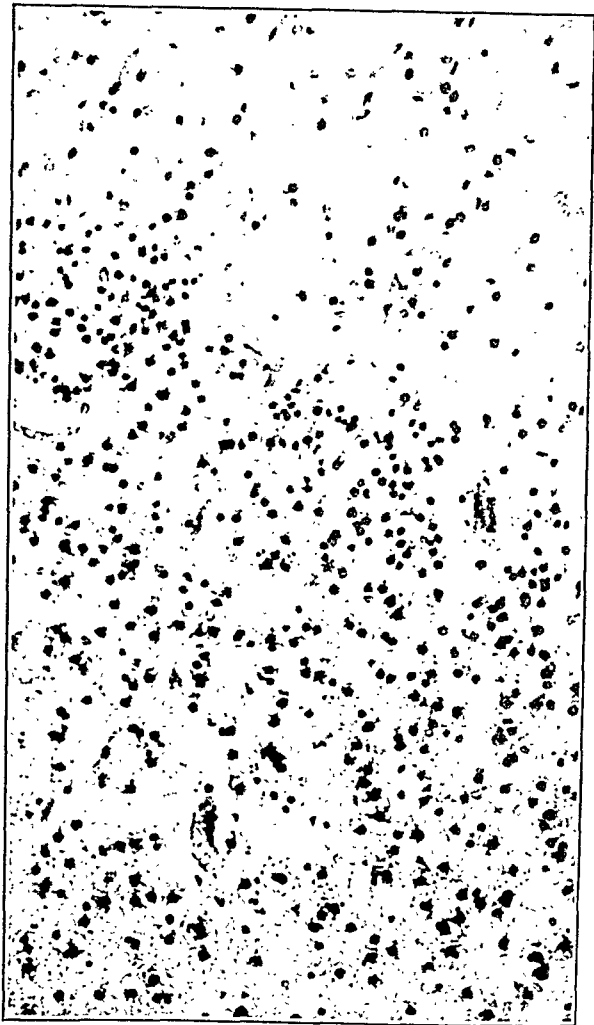
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## DESCRIPTION OF PLATES

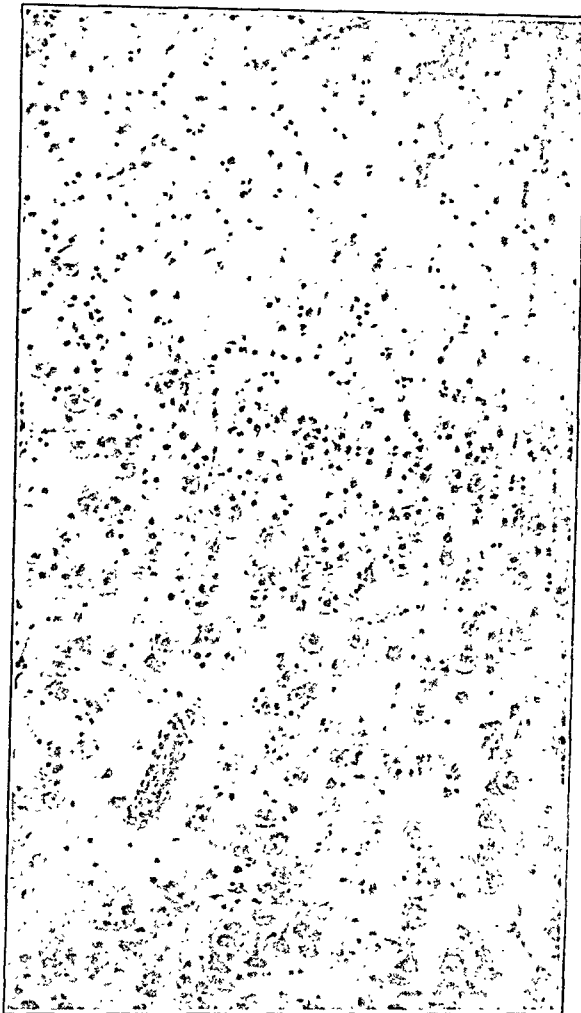
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### PLATE 33

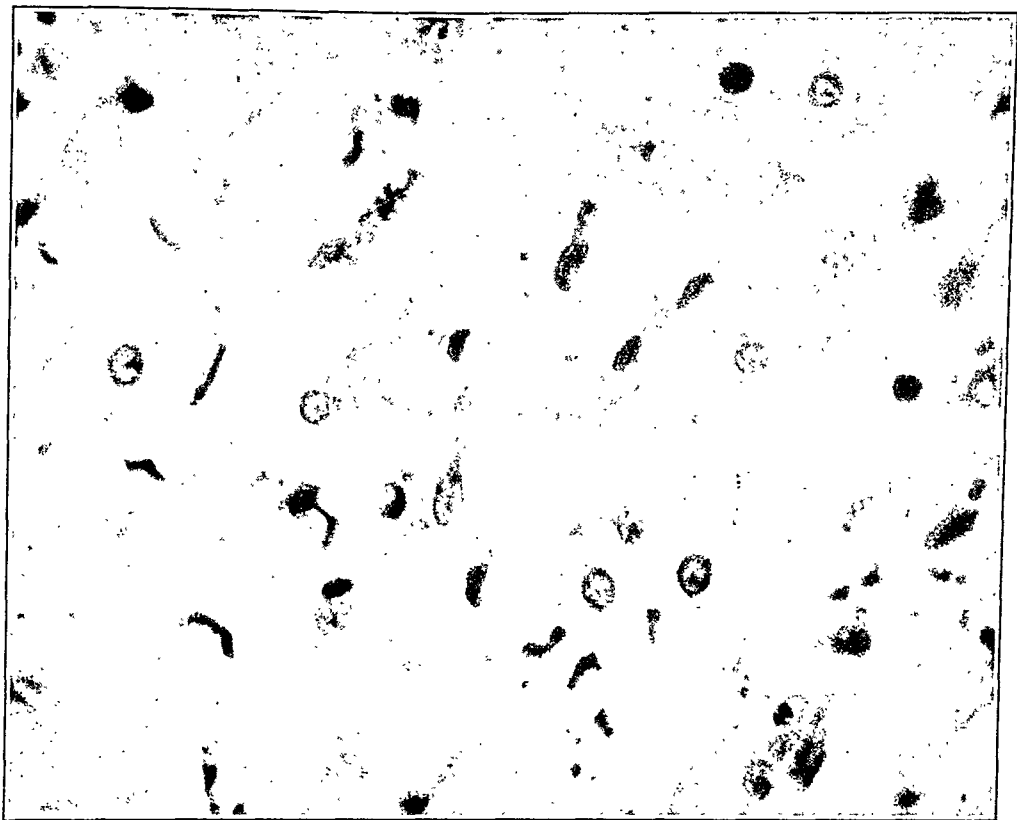
- FIG. 1. Poorly and irregularly stained cortical cells with postmortem dissolution of the cytoplasm. Child of 5 weeks with diarrhea and bronchopneumonia. Autopsy time 12 hours. Alcohol fixation. Case in Group 1, Division A.
- FIG. 2. Irregularly and poorly stained cortical cells with postmortem dissolution of the cytoplasm. Child of  $2\frac{1}{2}$  years, dying from kidney tumor and bronchopneumonia. Formalin fixation. Autopsy time 12 hours. Case in Group 1, Division C.
- FIG. 3. Microglia and macroglia showing increase in size and number in lower cortical layer. Child of 6 months, dying in convulsions 1 hour after second blood transfusion. Autopsy time 15 hours. Case 9, Group 1, Division C.



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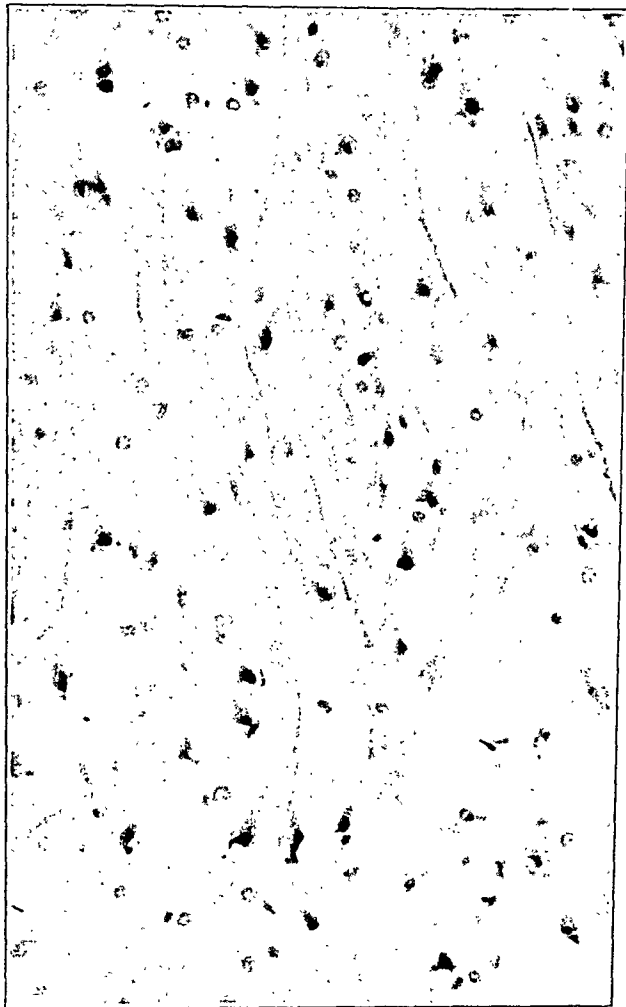
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PLATE 34

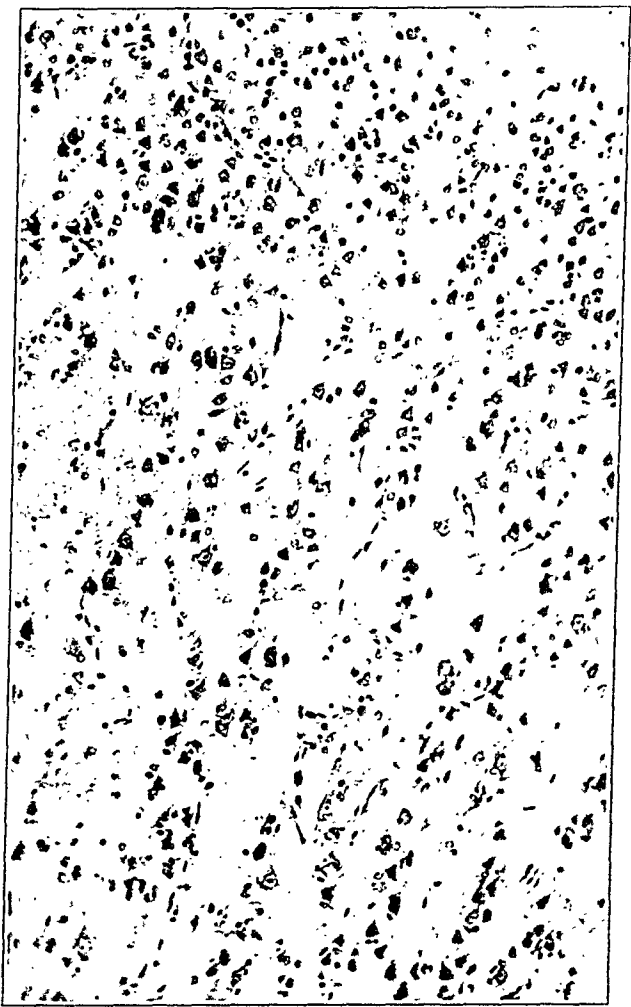
- FIG. 4. Bleaching of cornu ammonis and temporal cortex in child of 11 months. Death from whooping cough and convulsions. Alcohol fixation. Post-mortem time 7 hours. Case 5, Group 2, Division B.
- FIG. 5. Ischemic ganglion cells in Sommer sector of cornu ammonis. Child of 8 months, dying after 2 days of rash and convulsions. Alcohol fixation. Case 7, Group 2, Division B.
- FIG. 6. Case of lead poisoning of 5 months duration in child of 2 years. Cortical ganglion cells with distinctly stained cytoplasm and nuclei; also, some loss of ganglion cells and enlarged pale macroglia. Short fixation in formalin. Autopsy time 3 hours. Group 3.



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# THE AMERICAN JOURNAL OF PATHOLOGY

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VOLUME XII

MAY, 1936

NUMBER 3

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## COMPARATIVE CHEMICAL AND HISTOLOGICAL EXAMINATIONS OF AORTAS FOR CALCIUM CONTENT \*

### SERIES I

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In 1929 and 1930 we made a series of parallel chemical and histological tests for the metallic calcium content of human aortas. Experience soon taught us that single samples from the aortas of young persons gave uniform analyses and were satisfactory both for chemical and for microchemical examination, but that aortas from persons over 40 years of age, those that had undergone sclerotic changes, varied greatly from area to area so that no single sample was representative of the whole. We then took three samples from each aorta and arbitrarily chose segments from the arch, the thoracic portion and the abdominal portion. The chemical work and the histological examinations were done in separate laboratory departments and almost 50 aortas had been examined before comparisons were attempted. The results appeared to be beyond interpretation and the work was temporarily abandoned.

After reading Wells'<sup>1</sup> discussion of calcification of the aorta in Cowdry's "Arteriosclerosis," in which he said that "no one appears to have tried to correlate the chemical calcium content and the microscopic findings in the same aortas," we resumed the work.

\* Presented at the annual meeting of the American Association of Pathologists and Bacteriologists at Toronto, Canada, March 29, 1934.

Received for publication November 15, 1935.



## NATURE OF THE MATERIALS EXAMINED

The series included 52 aortas from persons varying in age from 2 months to 78 years. At autopsy the thoracic and abdominal viscera were removed, including the heart which was severed from the aorta just above the aortic valve cusps. The innominate, left carotid, left subclavian and iliac branches were then ligated and cut off with very short stumps. The aorta was opened longitudinally and a gross description made. The vessel was then removed and all fat and adventitial adhesions stripped away to the media. Chemical samples, 10 gm. when available, and pieces for microscopic study were then taken respectively from the arch, the thoracic and the abdominal portions, as already stated.

## CHEMICAL EXAMINATION

The calcium of the aorta was estimated by the method of Corley and Denis.<sup>2</sup> All samples for analysis were weighed with an error of not more than 0.5 per cent before appreciable evaporation had taken place. The opened aorta was flattened and the samples were then taken from about the sites of the histological specimens, as nearly as possible, equally from above and below these points.

The use of fresh material rather than the dried residue as a basis for the analysis simplified the procedure. At the same time it yielded figures that are more reasonably to be compared with microscopic findings since it avoids any concern with the relative rates of deposition of calcium and other tissue constituents.

The samples were placed in the tubes with the sodium hydroxide, sealed with tin-foil and saved until a sufficient number had been accumulated before heating under pressure. Corley and Denis recommend grinding the tissue. The omission of this step in no way changed the character of the solution. The tissue was completely disintegrated.

After digestion the solutions were acidified, diluted to a known volume and filtered through Jena fritted glass filters, thereby avoiding contamination from filter paper. In many instances there were stones present in the digestion tubes. These were dissolved and added to the solutions before filtration.

Duplicate aliquots of the filtered solutions were precipitated with

oxalate for the actual calcium determination and the procedure followed exactly as described. Blank determinations were made at intervals and were uniformly found to be too small to be significant. They were neglected in the calculation. The results were stated in mg. per cent of fresh tissue.

Numerous determinations of the composition of the calcifications have been made and are recorded in the literature. The minerals are deposited in proportions similar to those found in bone. Schönheimer<sup>3</sup> has recently made such a comparison. For our purpose the estimation of the calcium alone sufficed. The significance of the phosphorus determination would be complicated by the presence of varying and often large quantities of phosphatides.

### HISTOLOGICAL TECHNIQUE

Six pieces were taken from each aorta, duplicate pieces from each of the three segments being fixed in Zenker's fluid containing 5 per cent acetic acid and in 10 per cent neutral formalin.

The Zenker material was embedded in paraffin and cut in the usual way. The sections were stained with Mallory's phloxine-methylene blue stain, Weigert's elastic stain and Mallory's phosphotungstic acid hematoxylin. Zenker-fixed tissues were preferable for inflammatory changes and degenerations but were useless for estimating calcium content because the acetic acid removed the alkaline staining substances.

These stains were useful in comparing the sections with others removed at autopsy over a period of years. We had sometimes made the mistake of calling blue stained materials calcium but this practice was found to be in error, save in the instances of heavy calcium plates and areas of true bony metaplasia. None of them is a stain for calcium.

Formalin-fixed material\* was cut with the freezing microtome and was examined both for fatty changes and for calcium contents with Sudan III and hematoxylin, and for calcium deposits with

\* Formalin was neutralized with magnesium oxide for the general routine. Comparative tests were made by fixing pieces of infant's aorta after the routine method and in formalin kept over calcium carbonate. The specimens were washed in distilled water and stained with alizarin. Both sets were negative for absorbed calcium. After several months the amount of stainable calcium in atheromatous aortas kept in formalin was greatly decreased. Formalin becomes acid on standing and we interpreted the decrease as an evidence of acid decalcification.

Roehl's hematoxylin, von Kossa's silver method and Cameron's alizarin stain. All calcium stains were made in duplicate to exclude artifacts and extraneous precipitates and were mounted in glycerine jelly.

The Sudan III and hematoxylin stain was used to show, when possible, the relation between the various fatty changes as manifested by red or orange stained fat granules, crystals and globules, and the metallic salt deposits which included calcium. Macallum<sup>4</sup> in 1897, and later Wells<sup>5</sup> and Cameron,<sup>6</sup> showed that hematoxylin is not a specific stain for calcium but that it stains iron and chromium salts as well. While almost any of the alkaline salts may take the blue stain, iron is the most important of the group that may be mistaken for calcium.

In spite of the study of a great many preparations, we were unable to establish a consistent relation between the types of fatty change and the location of the metallic deposits. A few instances were seen where blue amorphous deposits were interspersed with fatty granules or globules. Other areas were found where large calcified plaques were surrounded by visible fat. Yet it was far from uncommon to find cysts with fat contents in which no calcium salts were stained and calcium and iron salt deposits with nothing in the way of visible fat.

*Roehl's Hematoxylin Stain for Calcium*<sup>4</sup>: This method has been recommended for the removal of the iron deposits, leaving calcium and one or two less important salts to be stained.

Frozen sections were washed thoroughly in distilled water, transferred to a 3 per cent solution of oxalic acid for  $\frac{1}{2}$  hour, washed and stained for about 1 hour in a ripened (3 months old) solution of hematoxylin in 50 per cent alcohol, and mounted in glycerine jelly.

We had little success with this method. The calcium plaques stained rather lightly. Some of the segments that gave high calcium values by chemical analysis were entirely negative in the stained sections. It was useful in showing that the salts which could be demonstrated occupied the same sites as the salts demonstrated with alum hematoxylin and von Kossa's stain.

*Von Kossa's Silver Stain*: This stain formerly was used as the routine method for the demonstration of calcium salts. Many modifications have been recommended and we tried several of them. Our best results were obtained in the following way:

Frozen sections were washed in distilled water and transferred to freshly prepared 10 per cent silver nitrate in open dishes. They were then placed in direct sunlight, out of doors and without the interposition of window glass for from  $\frac{1}{2}$  to 1 hour, depending on the season of the year. They were then washed in distilled water for at least  $\frac{1}{2}$  hour and counterstained lightly in phloxine and mounted in glycerine jelly. In the wintertime we exposed the section to an arc light instead of the sun, but the reaction was less intense.

Cameron<sup>6</sup> tested the action of silver nitrate on various salts in gelatin and reported that von Kossa's stain blackened calcium phosphates, carbonate, oxalate and oleate, also the salts of barium, strontium (ferric) iron, copper and magnesium.

Of all methods tested by us the von Kossa stain was, in spite of its non-specific action, the best index of the amount of calcium to be obtained by chemical analysis.

*Alizarin:* The amounts of calcium brought out with alizarin were relatively small and there was constantly a discrepancy between the amounts of stained calcium and that found by quantitative chemical analysis. When present the reactions were distinct and clear-cut.

By a series of experiments in which known quantities of salts were injected, Cameron<sup>6</sup> concluded that alizarin is differential for calcium with the exception of strontium, and that only freshly deposited salts of these metals are brought out in tissues.

Microscopic incineration was applied to several frozen sections and specimens were obtained that showed the location of the salts in the arterial walls. The test is not differential and discloses no more than von Kossa's stain.

*Summary of Technical Methods:* None of the methods shows all of the calcium salts present. Von Kossa's silver method most nearly paralleled the chemical analyses, in spite of the fact that it brings out ferric and other salts as well as calcium. Both Roehl's hematoxylin and alizarin fell far short of indicating the amount of calcium actually present.

#### COMPARISON OF CHEMICAL AND MICROCHEMICAL RESULTS

For the purpose of comparing the amounts of calcium found chemically with the amounts that could be shown by microchemical means in the various types of arteriosclerosis of the aorta, a protocol

was prepared for each case. These data included the name, age, color and sex of the patient, the clinical diagnosis, the serological reactions with reference to lues, and in parallel columns the diagnosis for each type of aortic lesion, the relative amounts of calcium shown in the sections, and the number of milligrams of metallic calcium per 100 gm. of wet aorta, found chemically, for each respective segment of each aorta. Seven apparently normal aortas from young persons gave an average of 22.8 mg. of calcium per 100 gm. of wet aorta.

The first step was to adopt a satisfactory nomenclature to be applied uniformly throughout the series.

### NOMENCLATURE

For some years it has been our practice to use Klotz' <sup>7</sup> classification of arteriosclerosis. However, Klotz' classification was devised for general arteriosclerosis and does not provide for some of the differentiations that seemed to us to be of advantage in our work. With only the lesions of the aorta in mind we adopted a descriptive terminology of our own as given below.

#### *Aortic Arteriosclerosis*

- (A) Intimal lesions
  - (1) Fatty streaking
  - (2) Nodular thickening (without cysts)
  - (3) Atheroma
    - (a) Grumous cysts
    - (b) Crystalline cysts
    - (c) Ulcerated cysts
  - (4) Visible calcification
    - (a) Diffuse
    - (b) Scaly plaques
    - (c) Heavy plates
- (B) Medial lesions
  - (1) Fatty changes
  - (2) Cystic degeneration
  - (3) Calcification
    - (a) Diffuse
    - (b) Plaque formation
- (C) Combined intimal and medial sclerosis
- (D) Aortitis
 

<ul style="list-style-type: none"> <li>(a) Exudative</li> <li>(b) Degenerative</li> <li>(c) Proliferative</li> </ul>	}	<ul style="list-style-type: none"> <li>Acute</li> <li>Rheumatic</li> <li>Luetic</li> </ul>
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### (A) *Intimal Lesions*

*Fatty streaking* was applied to the superficial intimal yellowish discolorations where the microscopic picture showed the fat to be intracellular or in pockets where the foam cell partitions had disappeared: six segments showing fatty streaking in persons under 40 years of age gave an average of 77.2 mg. of calcium and were all negative to calcium stains.

*Nodular thickenings* included the intimal nodular proliferations prior to cyst formation: ten segments averaged 168 mg.

*Atheroma* was used in the original sense of Haller (Klotz<sup>8</sup>) and limited to cystic changes in the intima. We included all cystic changes from early liquefaction necrosis to the typical cysts filled with "free fats, lipoids and cholesterol compounds." Some of the contents were amorphous or grumous, while others were crystalline and contained varying amounts of stainable calcium: fifteen segments averaged 298 mg.

*Visible calcification* of the intima was manifested in several degrees of intensity. Calcium occurred in diffuse granules in intimal nodules; in the form of very fine superficial scales just beneath the intimal surface; as definite plaques of varying size and thickness, some of which were firmly fixed while others were ulcerated; and as actual heavy plates which tore the tissues on removal and which occasionally had a supporting structure of metaplastic bone.

Owing to the way in which the samples were collected it was not possible to separate the intimal calcification histologically from the medial lesions. None of the segments could be interpreted as fairly representing an entire aorta.

### (B) *Medial Lesions*

Changes in the aortas were rarely limited to the media, although instances were found where the medial lesions were predominant at the levels examined: thirteen such segments averaged 345 mg. of calcium and all gave positive silver stains.

The earliest changes observed were brought out by the positive Sudan III staining of the smooth muscle cells. Calcium granules were sometimes found in these degenerated muscle cells and sticking to the elastic fibrils between them.

Cysts were found in the medias of some aortas unassociated with those in the intima. Their contents varied from grumous to crystalline: eight segments showing ulcerated crystalline cysts averaged 770 mg. of calcium.

Calcium salts in stainable forms were manifested in three degrees of intensity: as sparsely distributed granules clinging to the elastic tissue fibrils; as bands varying in width from narrow streaks lying just beneath the internal elastic lamina to broad, dense, unbroken zones involving more than half of the medial thickness; and as concentrated masses in plaque form.

### (C) *Combined Intimal and Medial Plates*

In the more advanced examples of arteriosclerosis practically every aorta presented combinations of any or all of the lesions mentioned above, and they were rarely limited sharply to the intimal or medial layers but extended from one to the other. Separate lesions of the intima and media were sometimes found near each other, but without apparent relation. Thirteen segments containing combined intimal and medial plates averaged 1009 mg. of calcium.

### (D) *Aortitis*

Several examples of aortitis were encountered: these varied from acute leukocytic exudates in the intima, as seen in a case of septicemia, to widespread degenerations of the muscle cells and elastic tissue accompanied by proliferation of connective tissue and vascular invasions from the adventitia. The latter were examples of luetic and rheumatic aortitis. In all cases in this group there was considerable overlapping of exudative, degenerative and proliferative processes. Three of the cases presented numerous calcium plates in addition to aortitis. One such segment reached 957 mg. of calcium. As a rule the examples of aortitis gave lower calcium values than the average for the age group in which they occurred. The average calcium value for 10 cases of aortitis was 337 mg.

The calcium content in relation to types of lesions as shown above was inconsistent. Not only did similar lesions vary in the amounts recovered chemically but there were wide variations between similar lesions from patients of different ages and in the segments from vari-

ous levels of the same aorta. Since the results seemed to depend largely on the ages of the patients, we tabulated the amounts obtained chemically according to decades into eight groups.

### GROUP I. CHILDREN UNDER 10 YEARS OF AGE

The first group included 6 aortas from children under the age of 10 years (Table I). Microchemical methods failed to demonstrate calcium in any aortas of this group. Three were grossly and microscopically negative. A fourth presented simple fatty streaking. Two were thickened and inelastic. The outstanding one of the group was from a child with rickets which yielded 114 mg. of calcium by chemical analysis.

TABLE I

*Group I. Birth to 10 Years*

Autopsy No.	Age	Sex	Color	Average* mg.	Gross examination	Cause of death
A-29-85	5 mo.	F	B	35.0	Degenerative aortitis	Congenital lues
A-29-89	1 yr.	M	W	37.0	Fatty streaks	Hydrocephalus
A-29-90	1 yr.	M	W	12.5	Negative	Acute infection
A-29-101	2 mo.	F	B	114.0	Thickening in gross	Rickets and diphtheria
A-30-20	19 mo.	F	W	25.0	Negative	Polyglandular dystrophy
A-30-27	10 yr.	M	W	16.3	Negative	Auto accident

40 = Average of 6 — Birth to 10 years

\* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

### GROUPS II AND III. 11 TO 21 YEARS, AND 21 TO 30 YEARS

Groups II and III included 3 cases between the ages of 10 and 20 years, and 3 between 20 and 30. As shown in the tables, 4 aortas were negative in the gross and 2 presented simple fatty streaking. No calcium was shown by microchemical methods in any of them. With the exception of the 25 year old patient with chronic tuberculosis, the calcium content was fairly uniform.

### GROUP IV. 31 TO 40 YEARS

In this group there were 8 aortas that presented a variety of pathological changes. Three showed nothing more in the gross than fatty intimal streaking. In these the microchemical tests were negative



and an average for all nine segments gave a chemical analysis of 95 mg. of metallic calcium. Two presented nodular intimal thickenings which were negative to all microchemical tests except silver, the silver reaction being brown rather than black. Three presented very definite aortitis, and of these 2 were characteristic of lues. The aorta from case A-29-86 was dilated and thickened in the first portion. Microscopically the elastic and muscular layers were replaced by connective tissue. The microchemical reactions were

TABLE II  
*Group II. 11 to 20 Years*

Autopsy No.	Age	Sex	Color	Arch *	Thoracic*	Abdominal*	Average *	Gross examination	Cause of death
	<i>yrs.</i>			<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>		
A-29-72	17	F	W	25.6	..	..	25.6	Negative	Tuberculosis
A-29-80	18	F	W	..	28.4	29.0	28.7	Negative	Endocarditis
A-29-100	13	F	W	..	14.0	..	14.0	Negative	Paratyphoid fever

22.8 = Average of 3 — 11 to 20 years

TABLE III  
*Group III. 21 to 30 Years*

Autopsy No.	Age	Sex	Color	Arch *	Thoracic*	Abdominal*	Average *	Gross examination	Cause of death
	<i>yrs.</i>			<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>		
A-29-89	22	M	W	43.0	40.0	29.0	37.3	Negative	Pneumonia
A-29-102	28	M	W	69.0	56.0	50.0	58.3	Fatty streaks	Septicemia
A-30-12	25	M	W	62.0	60.0	72.0	64.6	Fatty streaks	Tuberculosis

53.4 = Average of 3 — 21 to 30 years

\* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

negative and the metallic calcium low. In the thoracic segment two processes were found—aortitis and cystic atheroma. Microscopically the cysts were filled with crystalline material and calcium granules. The von Kossa stain brought out a moderately heavy granular deposit in the media. The calcium content by chemical analysis, 853 mg., appeared to be out of proportion and greatly in excess of the other changes (see Table IV). In this age group the thoracic segments gave the highest calcium values.

TABLE IV

## Group IV. 31 to 40 Years

Autopsy No.	Age	Sex	Color	Arch †	Thoracic †	Abdominal †	Average †	Gross examination	Cause of death
	yr5.			mg.	mg.	mg.	mg.		
A-29-86	38	F	W	90.0	853.0	131.0	358.0	Atheromatous aortitis	Lues and alcoholic cirrhosis
A-29-92	38	F	W	118.0	253.0	166.0	179.0	Aortitis	Streptococcus cellulitis
A-29-94	31	F	W	108.0	103.0	61.0	90.6	Fatty streaks	Pneumonia
A-30-25	38	M	W	*	258.0	*	258.0	Luetic aortitis	Cerebral hemorrhage
A-30-33	36	M	W	209.0	133.0	178.0	173.3	Moderate thickening	Pneumonia
A-30-34	37	M	W	88.0	124.0	98.0	103.3	Fatty streaks	Pneumonia
A-30-38	35	M	W	130.0	101.0	63.0	98.0	Fatty streaks	Acute alcoholism
S-34-193	35	M	W	66.0	100.0	77.0	81.0	Nodules	Pneumonia
				115.5	240.6	110.5	167.6 =	Averages of 8 — 31 to 40 years.	

\* Segments lost in autoclave.

† The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

## GROUP V. 41 TO 50 YEARS

Seven of the 11 cases in this group had an average age of 42 years and an average calcium content by analysis of 214 mg. They presented little contrast in calcium content from segment to segment (see Table V). In the remaining 4 cases the mean age was 48 years and the average calcium content 270 mg. Microchemically calcium was demonstrated in the aortas from cases A-29-74, A-29-103, A-30-2, A-30-24 and A-30-37. In case A-29-96, where the picture was that of luetic aortitis and the average calcium content was 374 mg., no calcium was shown microchemically by any method. This apparently inconsistent result indicates that the quantity of calcium present is independent of its visibility. In this group the highest calcium values were in the abdominal segments.

## GROUP VI. 51 TO 60 YEARS

All of the aortas in this group presented arteriosclerotic changes and calcium was stained in all except cases A-29-76 and A-30-6. The amounts of calcium obtained varied more widely than in any other age group. The lowest amount was recovered from case A-29-76, where the average for the three segments was 153.6 mg. This patient was a man 50 years of age with typical luetic aortitis, who died from a cerebral hemorrhage. Three cases A-29-97, A-29-106 and A-30-8 presented widespread combined lesions including atheromatous cysts, diffuse medial calcification and isolated calcium plaques or plates. All averaged over 1100 mg. of metallic calcium. In the first sections the abdominal segment from case A-30-8 showed 2312 mg. of calcium and only atheromatous cysts with granular calcium and crystalline material. As it was the only instance of excessively high calcium without plates, the original formalin-fixed material was re-examined and definite plates found. Two of the 10 cases were 60 years of age and barely escaped the 61 to 70 year group. These cases averaged well over 1100 mg. of calcium. In this group the highest average calcium content was found in the abdominal segment.

## GROUP VII. 61 TO 70 YEARS, 1 CASE 78 YEARS OF AGE

The calcium content of the respective aortas in this group was higher than in the group between 51 and 60 years of age, but the

TABLE V  
Group V. 41 to 50 Years

Autopsy No.	Age	Sex	Color	Arch †	Thoracic †	Abdominal †	Average †	Gross examination	Cause of death
	yrs.			mg.	mg.	mg.	mg.		
A-29-71	42	M	W	257.0	178.0	*	217.0	Aortitis	Gangrenous lung
A-29-74	50	M	W	241.0	440.0	795.0	492.0	Atheromatous cysts	Septicemia
A-29-83	42	M	W	139.0	192.0	137.0	156.0	Nodular thickening	Carcinoma
A-29-96	42	M	B	330.0	413.0	380.0	374.0	Aortitis	Lues, pneumonia
A-29-103	47	M	W	215.0	150.0	106.0	157.0	Atheromatous cysts	Exophthalmic goiter
A-30-2	43	M	W	201.0	209.0	324.0	245.0	Intimal plaque	Lung abscess
A-30-14	48	M	W	130.0	219.0	110.0	153.0	Aortitis	Arthritis, pneumonia
A-30-24	43	M	W	314.0	324.0	242.0	293.0	Combined intimal and medial cysts	Cardio-renal
A-30-28	41	M	B	84.0	66.0	55.0	68.0	Nodular thickening	Gangrenous lung
A-30-37	47	M	B	*	250.0	307.0	278.0	Atheroma and aortitis	Pneumonia
A-30-39	41	M	B	153.0	104.0	145.0	134.0	Negative	Pneumonia
				206.0	231.4	260.0	233.4	= Averages of 11 — 41 to 50 years.	

\* Segment lost.

† The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

TABLE VI  
Group VI. 51 to 60 Years

Autopsy No.	Age	Sex	Color	Arch*	Thoracic*	Abdominal*	Average*	Gross examination	Cause of death
A-29-76	yrs. 50+	M	W	mg. 114.0	mg. 121.0	mg. 226.0	mg. 153.6	Cyst and aortitis	Cerebral hemorrhage
A-29-78	54	M	W	182.0	213.0	923.0	439.3	Atheromatous cysts	Alcoholic cirrhosis
A-29-97	60	M	W	1148.0	1175.0	1067.0	1130.0	Medial plaques	Chronic vegetative endocarditis
A-29-106	60	M	W	351.0	1265.0	1926.0	1180.0	Atheromatous cysts	Chronic cardio-renal
A-30-5	58	M	W	475.0	355.0	418.0	416.0	Luetic aortitis	Chronic cardio-renal
A-30-6	55	F	W	493.0	193.0	466.0	384.0	Atheromatous cysts	Hodgkin's disease
A-30-8	57	M	W	508.0	718.0	2312.0	1179.0	Atheromatous cysts	Septicemia
A-30-15	53	M	W	396.0	399.0	320.0	372.0	Nodular thickening	Sarcoma
A-30-31	51	M	W	180.0	512.0	71.0	254.0	Atheromatous cysts	Septicemia
A-30-32	50+	M	W	111.0	130.0	957.0	399.3	Atheroma and plates	Lues
				395.8	508.1	868.6	590.8	= Averages of 10—51 to 60 years.	

\* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

TABLE VII

Group VII. 61 to 70 Years, Including 1 Case, Age 78 Years

Autopsy No.	Age yrs.	Sex	Color	Arch*	Thoracic*	Abdominal*	Average*	Gross examination	Cause of death
A-29-81	63	M	W	mg. 313.0	mg. 133.0	mg. 420.0	mg. 289.0	Atheromatous cysts	Pneumonia
A-29-95	66	M	W	314.0	405.0	694.0	471.0	Atheromatous cysts, aortitis	Sarcoma (lues)
A-29-99	70	M	W	919.0	1644.0	930.0	1164.0	Ulceration, plate	Cerebral hemorrhage
A-30-7	66	M	W	523.0	492.0	1348.0	788.0	Atheromatous cysts	Cirrhosis
A-30-16	78	M	W	1135.0	716.0	1144.0	998.0	Atheromatous cysts and plates	Nephritis and diabetes
A-30-19	62	M	W	121.0	263.0	983.0	456.0	Ulceration, plaques	Pneumonia
A-30-21	67	M	W	426.0	340.0	1068.0	611.0	Atheromatous cysts and plates	Pneumonia
A-30-26	67	M	W	223.0	368.0	316.0	302.0	Nodular intima	Accident
S-34-190	61	M	W	288.0	200.0	512.0	333.3	Atheromatous cysts and plaques	Cardio-renal
S-34-191	61	F	W	1050.0	420.0	800.0	757.0	Diffuse medial and plaques	Burns
S-34-192	64	M	W	392.0	160.0	520.0	357.0	Luetic aortitis	Cardio-renal

518.5      467.3      794.0      593.2 = Averages of 11 — 61 to 70 years; and 1 aorta from a patient 78 years of age.

\* The figures in this column represent metallic calcium in mg. per 100 gm. of wet aorta.

total average was not as high because there were 3 aortas with unusually high calcium content in Group VI. Practically all of the segments presented combined atheromatous cysts and diffuse medial disease with plaques and plates. All of the aortas contained stainable calcium. Again, the heaviest calcium deposits were found in the abdominal segments just above the bifurcation.

There was 1 aorta from a patient over 70 years of age in the series. It presented advanced arteriosclerosis with both intimal and medial changes and is tabulated in Group VII.

### DISCUSSION

A summary of the results by decades is shown in Table VIII. It is apparent that changes occur in the aortas during the course of life which lead to an accumulation of calcium, the total amounts of which exceed that of the bodily tissues generally. The affinity of the tissues of the aorta in advanced years for calcium is greater than that of the circulating blood.

A number of theories have been suggested to explain this excess of calcium. None has been altogether satisfactory, but since there are so many and varied histological changes in the vessel it is probable that multiple factors are responsible. Wells<sup>1, 9</sup> called attention to the fact that living and dead colloids behave similarly; fresh gels contain much water and, as age increases, the capacity to retain it decreases and eventually the gel becomes granular. The elastin in the aortic walls very likely behaves in this way, and he further compared the process to the gradual hardening of rubber tubing. He cited hyaline cartilage as another example of a colloid that loses its elasticity and takes up calcium. Gazert<sup>10</sup> reported that sclerosis and calcium increased as the proportion of nitrogen decreased. Klotz<sup>11</sup> explained the increase in calcium in atheromatous cysts as a calcium replacement of soluble salts in the soaps formed during the degeneration of fatty substances. Wells,<sup>1, 5, 9</sup> Schönheimer,<sup>3</sup> and others, have raised many objections to Klotz' views. It is certain that we recovered large amounts of masked calcium and demonstrated calcium deposits in the aortas of our series where no previous fatty changes could be brought out.

Whatever the nature of the calcification process of aortas, the ratio of calcium phosphate to calcium carbonate appears to be

similar to that of bone. This has been shown by Barillé,<sup>12</sup> in 1910, and by Schönheimer,<sup>3</sup> in 1928. The generally accepted ratio is given as calcium phosphate 88 to 90 per cent, carbonate 10 to 12 per cent, and magnesium salt 1 to 1.5 per cent.

### *Calcium Content According to Type of Lesion*

The results of our efforts to correlate the calcium recovered chemically with the types of lesion were unsatisfactory. It was soon apparent that moderate intimal lesions existed without an increase

TABLE VIII

*Average Calcium Content by Decades per 100 gm. of Wet Aorta*

Decades	No. cases examined	No. positive microscopically*	High †	Low †	Average †
<i>yrs.</i>			<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Birth to 10 .....	6	0	114.0	12.5	40.0
11 to 20 .....	3	0	28.7	14.0	22.8
21 to 30 .....	3	0	64.6	37.3	53.4
31 to 40 .....	8	1	358.0	81.0	167.6
41 to 50 .....	11	5	492.0	68.0	233.4
51 to 60 .....	10	8	1180.0	153.6	590.8
61 to 70 .....	10	10	1164.0	289.0	552.7
71 to 80 .....	1	1	998.0	998.0	998.0

\* Includes all cases where Roehl's hematoxylin, alizarin and von Kossa's stains were positive.

† The high and low calcium columns show the high and low calcium contents obtained by averaging three segments from the same aorta, not the amount found in the highest or lowest segment analyzed. The average in the last column represents all of the aortas for each age group.

in calcium, chemically, and that concentrated deposits in the nature of plaques and plates gave the highest amounts. Of eleven examples of luetic aortitis only two contained an excess of calcium, while the average content was lower than that for the age group in which it occurred.

We realized that there are additional factors responsible for our failure to obtain comparable figures for similar lesions. Some of these factors we know are beyond our control while others we think may be brought out by more carefully controlled data and these will be discussed in a later paper.

### CONCLUSIONS

Fifty-two aortas from patients between the ages of 2 months and 78 years were analyzed chemically for metallic calcium and the



results compared with the microchemical tests for calcium salts on the same aortas with the conclusion that:

(1) None of the microchemical tests recommended for visible calcium gives more than a vague idea of the amount that can be recovered from the same aorta chemically. Von Kossa's silver method is not a specific stain for calcium, but it is the most satisfactory microscopic indicator of the comparative amounts of calcium deposited in sclerotic lesions.

(2) Calcium deposits were brought out microchemically in only 1 of 20 aortas from cases under 40 years of age, in 75 per cent of aortas from cases over 40 years of age, and in 100 per cent of those over 60 years.

(3) As age advances there is a consistent increase in the calcium content of aortas in excess of that of the bodily tissues generally.

(4) Mild intimal lesions may occur without increased calcium by chemical analysis.

(5) When the segments of all aortas from cases over 40 years of age are averaged, the heaviest calcium deposits are found in the abdominal portions.

(6) In advanced aortic arteriosclerosis there is constantly found to be an overlapping of "type lesions," such as atheromatous cysts, diffuse medial calcification and plaque deposits. In this of all groups of lesions, the greatest inconsistencies between chemical and microscopic results occur.

(7) In very advanced sclerotic lesions, characterized by heavy plate-like calcium deposits, the chemical analyses yield the highest calcium values and the amounts are most nearly consistent, when any 2 given sclerotic aortas are compared.

(8) If the results obtained by chemical analysis are to be correlated with calcium, which can be shown microscopically in sclerotic aortas, additional knowledge is required.

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# THE CALCIUM CONTENT OF ARTERIOSCLEROTIC AORTAS \*

## SERIES II

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In the report <sup>1</sup> of our first series of parallel chemical and microscopic examinations for calcium in the aortas of 52 human cases we showed that there is a gradual increase in the calcium content by decades and that after the age of 40 years the calcium deposits are unequally distributed along the vessel. As stated in our previous report, when no gross or microscopic evidences of calcification were present the chemical analyses were constant within reasonable limits for aortas of similar ages, but when sclerosis was present samples taken from two or more levels of the same aorta varied widely in the amounts of calcium recovered. Well developed and easily stained calcium plaques regularly gave high calcium values which were comparatively constant. The intermediate groups (*a*) of diffuse medial sclerosis and (*b*) of atheromatous cysts yielded such wide variations chemically that we were unable to correlate our results with the data at hand. It was in an effort to interpret the lesions in the latter two groups that we determined to make additional studies.

Since calcification of the media seems to be related primarily to changes in the elastic tissue, while the deposit of calcium in atheromatous cysts follows necrosis and fatty changes beginning in the intima, we are discussing them under separate headings.

### MEDIAL CALCIFICATION

The evidence derived from previous studies indicates that medial calcification is associated with the effects of age on the elastic tissue elements. The aorta belongs to the group of elastic arteries and has a proportion of elastic tissue equal to, or greater than, the amount of smooth muscle present (Klotz <sup>2</sup>). The elastic layers alternate with the muscle layers and are bound to each other by connecting

\* Received for publication November 15, 1935.

bridges so that compact expansile walls are formed. Wells<sup>3</sup> states that elastin, the characteristic substance of elastic tissue, is a gel and discusses its probable chemical nature. It appears to resemble other gels in its physical behavior and to lose water with age. He quoted Selig who reported on the elastin content of normal and sclerotic arteries. Elastin decreased from 46-48.17 per cent in normal aortas to 10.76 per cent in the most atheromatous, while the fatty matter increased from 3.66-4 per cent to 14.58 per cent, respectively, in the same group of vessels. Wells likened the loss of elasticity of the aorta to the hardening of old rubber tubing, and the deposit of calcium in the elastic layers to the calcification of cartilage in the formation of bone.

We made no analyses for elastin but three of our observations seemed to support the theory that medial calcification depends on the state of the elastic tissue fibrils. (a) The earliest visible evidence of calcium in the media appeared in the form of alizarin stained granules resting on the elastic fibrils where no fatty changes were observed in the intervening smooth muscle. (b) Early fatty changes, brought out by Sudan III, were seen in strands of smooth muscle in several specimens where no stainable calcium was found. This seemed to show that the two processes could go on independently. (c) In a series of aortas in which a great deal of elastic tissue had been destroyed, owing to luetic aortitis, the arteries were generally lower in chemical calcium values than uncomplicated sclerotic aortas in similar age groups. We interpreted the relatively low calcium content to the loss of elastin.

It is generally recognized that every aorta contains more calcium by analysis than can be shown by staining methods, yet it seemed to us that if the process consisted in the simple taking up of calcium salts by a colloid substance, the width of the stainable medial band should indicate in a general way how much calcium could be recovered chemically. By striking an average of the analytical results for a series of aortas in which the most obvious lesion was that of medial calcification, and using the average as a basis for comparison, we found that certain members of the group fell below the expected amount and others exceeded it considerably. Occasionally one segment of a given aorta was found to contain calcium out of proportion to its other fractions. Making all allowances for the non-specific nature of von Kossa's stain, it helped, in a measure, to

explain the chemical variations. The amounts brought out by the stain varied from a few granular deposits to solid black bands extending through at least half of the medial coat. Sections from three levels of the same aorta showed that the width of the bands regularly varied from level to level and therefore chemical variations within certain limits were to be expected.

In addition to medial calcification we found that allowances had to be made for complicating atheromatous cysts. In Series I no allowance was made for such cysts unless one of them occurred in the section under examination. In Series II the whole segment was surveyed for atheromatous changes and there was not a single example found of diffuse medial calcification which was not complicated by atheroma. In view of the facts we believe the following conclusion is justified: In trying to correlate the results of microscopic and chemical examinations of aortas having medial calcification as their chief lesion, allowances have to be made both for variations in the width of the medial calcium band and for possible atheromatous cysts somewhere in the segment.

#### CALCIUM DEPOSITS IN ATHEROMATOUS CYSTS

Calcium is increased in both visible and masked forms in atheromatous cysts. According to Ophüls<sup>4</sup> the essential steps in the pathogenesis of an atheromatous cyst are as follows: (1) Fatty changes occur which are at first confined to the intima. Later the muscle cells of the upper part of the media become involved, and still later the cells disintegrate and permit a part of the contents in the form of fat droplets to be dispersed throughout the interstitial tissues. (2) Dense fibrous tissue develops in the intima over the primary lesion and this in turn undergoes a similar set of degenerative fatty changes. (3) The resulting necrotic material is at first finely granular. Chemically it contains free fats, fatty acids, cholesterol and calcareous material. The readily soluble constituents disappear, the cholesterol remains in the form of crystals and the calcareous material persists in granular form. (4) The degeneration cysts continue to increase in size and later may either ulcerate and discharge their contents into the blood stream, or become permanently sealed off by the formation of solid calcium plates.

Recently Rosenthal (1934)<sup>5</sup> has shown that the fat content of aortas increases with the age of the patient and runs an increase by decades, which in a general way parallels the increase of calcium found by chemical analysis in our series. While we found many instances of visible calcium deposits in atheromatous cysts along with fat staining materials and cholesterin crystals, we did not find any constant proportional relation between the two varieties of substances. The results of analysis for calcium, whether high or low, could not be predicted in atheromatous cysts by the amounts seen in the stained sections.

Summarizing, it appears that metallic calcium in atheromatous cysts is associated with lipoid degeneration and the amount depends on the nature of the degenerative products and their affinity for calcium at any given time. In the average sclerotic aorta both intimal cysts and medial hardening are present, both tend to increase the amount of calcium present, and the size, number and extent of each influences the amount of calcium contained.

## ANALYSIS OF SERIES II

In the last 4 aortas of the first series gross photographs were taken with threads laid across them to mark the sites of the microscopic specimens. It was from these photographs that we realized the influence that the immediate environment might have in explaining the differences in microscopic and chemical analyses. We decided to select 11 additional aortas and submit them to exceptional scrutiny before analyzing them. Instead of taking consecutive cases we selected the second series with reference to the kind of lesions they contained. They were photographed immediately after removal from the body, the sites of the microscopic sections were marked and careful detailed gross descriptions made. All of the chemical analyses were made by one of us and all of the microscopic technical work was done by the other. The analyses were made by the Corley-Denis Method<sup>6</sup> and reported in mg. per 100 gm. of wet aorta, just as in the first series. The technical work was done entirely on frozen sections, except in 2 cases of aortitis where paraffin sections were also made. The stains used were von Kossa's silver stain, alizarin after the technique of Cameron, and Sudan III and hematoxylin. The methods were applied in accordance with the techniques previously reported.<sup>1</sup>

A two-page protocol was prepared for each aorta. Beginning on the left-hand side of the first page there was placed a gross photograph of the aorta with the sites of the microscopic samples marked. In the second column was a synopsis of the gross description opposite its respective segment. In the third column was a rough sketch of the silver reaction showing the kind, size and location with reference to coats of all calcium deposits found. In the fourth column was a similar sketch in blue, red and black, which recorded the Sudan III reaction. The alizarin stains were merely used as controls since they stained only part of the known calcium present. Roehl's hematoxylin was discarded in the second series. In the fifth column were placed the results of the chemical analysis for each level. On the second page was a complete gross and microscopic record.

### GROUP I

Table I shows the results of the analysis of 7 aortas from patients under 60 years of age, and places side by side the results of the gross, microscopic and chemical examinations of each segment. In cases A-34-36 and S-34-193 the patients were both under 40 years of age; the microscopic lesions were of the type of fatty streaks limited to the intima; neither patient had been chronically ill and the calcium values for all segments were low. Case A-34-8 will be discussed with the group of aortitis lesions. In case A-34-23 the Sudan III preparations from all three segments were similar and there was a gradual increase in diffuse medial calcification from the arch to the bifurcation, but here it was necessary to consult the gross findings to see why there was more than twice as much calcium in the abdominal segment as there was in the arch. The first segment in case A-34-32 presented an unusually high calcium content and the protocol shows that it contained atheromatous cysts with crystalline contents, one large calcium plate, and heavy diffuse medial calcification.

In contrast, the third segment of case A-34-40, which had 2408 mg. of calcium, the highest in the group, had numerous calcium plates, nine of them large enough to be counted, as well as cysts filled with grumous contents and medial calcification. This group illustrates well how many factors must be taken into account before the microscopic and chemical findings can be correlated.



TABLE I

Ages 28 to 60 Years Inclusive

Case No.	Classification of segment*	Age	Sex	Color	Calcium	Gross examination	Silver reaction in media	Intima	Cause of death
A-34-36	(b) (b) (b)	yr. 28	F	W	mg. 69.0 74.0 93.0	Fatty streaks " " " "	Negative " "	Foam cell nests " " " "	Bichloride poisoning
S-34-193	(b) (b) (b)	35	M	W	66.0 100.0 77.0	Fatty streaks " " " "	Negative " "	Foam cell nests " " " "	Pneumonia
A-34-8	(g) (h)	49	M	W	309.0 72.0	Luetic aortitis " "	Negative " "	Aortitis Atheromatous cysts and aortitis Negative	Cerebral accident
A-34-23	(a) (d) (e)	60	F	W	48.0 242.0 444.0	Negative Superficial scales Atheromatous cysts and plaques Small plaques	Light Moderate " "	Grumous cysts " " " "	Peritonitis
A-34-32	(f) (e) (e) (f)	57	M	W	492.0 1144.0 572.0 724.0	Atheromatous cysts and plates Inelastic " "	Heavy " " " "	Crystalline cysts and plates Grumous cysts Large plate	Pneumonia
A-34-37	(c) (d)	60	F	W	233.0 370.0 303.0	Scales Plaques and atherom- atous cysts Atheromatous cysts	Light " " " "	Grumous cysts Crystalline cysts " " " "	Cerebrospinal lues
A-34-40	(c) (d) (e) (f)	51	M	W	220.0 176.0 2408.0	Inelastic Cysts and small plaques Numerous plates	Moderate " " " "	Grumous cysts Crystalline cysts Cysts and plates	Nephritis

\* The small letters in this column show where the segment is classified for calcium content by lesion, in Table IV.

TABLE II  
Ages 61 to 75 Years Inclusive

Case No.	Classification of segment*	Age	Sex	Color	Calcium	Gross examination	Silver reaction in media	Intima	Cause of death
S-34-190	(h) (h) (i)	61 yrs.	M	W	288.0 200.0 512.0	Nodular thickening " Medial plates	Light " Heavy	Atheromatous cysts and aortitis Aortitis Medial plates	Heart disease
S-34-191	(e) (d) (f)	61	F	W	1050.0 420.0 800.0	Atheromatous cysts and plates Many plaques Intimal and medial plates	Heavy Moderate Heavy	Grumous cysts and plates Grumous cysts Crystalline cysts	Accidental
S-34-192	(c) (c) (e)	64	M	W	392.0 160.0 520.0	Ulcerated atheromatous cyst Scales Ulcerated plates	Light Negative Heavy	Crystalline cysts " Cysts and plates	Cardio-renal
A-34-22	(c) (c) (e)	63	M	W	104.0 127.0 368.0	4 atheromatous cysts Small atheromatous cysts Small plates	Negative " Moderate	Grumous cysts Intracellular fat Cysts and small plaques	Addison's disease
A-34-24	(d) (d) (c)	68	M	B	226.0 432.0 287.0	Intimal cysts Atheromatous cysts "	Moderate Heavy Moderate	Crystalline cysts " Grumous cysts	Tuberculous nephritis
A-34-39	(h) (i)	64	F	W	Lost 161.0 610.0	Atheromatous cysts Nodules Plates	Negative " Heavy	Crystalline cysts and aortitis Grumous cysts and aortitis Plates and aortitis	Myelogenous leukemia and luetic aortitis
A-34-25	(f) (d) (f)	75	F	W	1056.0 327.0 2290.0	Ulcerated cysts Scales Ulcerated plates	Heavy " "	Crystalline cysts " Plates	Diabetes
A-34-28	(g) (g) (g)	75	M	B	220.0 178.0 191.0	Luetic aortitis " "	Light " Heavy	Aortitis " "	Lues

\* The small letters in this column show where the segment is classified for calcium content by lesion, in Table IV.

## GROUP II

In Group II there were 8 aortas from patients over 61 years of age, and all of them showed more or less arteriosclerosis. Of the three segments containing the highest calcium values, two combined atheromatous cysts with heavy diffuse medial calcium deposits and isolated calcium plates. The other had heavy medial calcification and both intimal and medial plates. Of the aortas having the lowest three calcium values 2 were from cases of luetic aortitis and 1 was from a case of Addison's disease with an exceptionally well preserved aorta. While more uniform results than those in the preceding group were obtained, this series of cases also emphasized the important factor of careful gross examination.

*Analysis of Results*

In the examination of the aortas in Series II we believe that every possible precaution was taken in the selection of the samples and in the performance of the tests. Wide variations in calcium values still occurred.

We have tabulated the amounts of calcium according to decades separately for Series I and II, and for the total of the 63 aortas.

TABLE III

*Average Amounts of Calcium by Decades. Metallic Calcium in mg. per 100 gm. of Aorta*

Decade	Series I	Average	Series II	Average	Total	Total average
yr.		mg.		mg.		mg.
under 10 . . . .	6 cases	40.0	0 cases	0	6 cases	40.0
11 to 20 . . . .	3 "	22.8	0 "	0	3 "	22.8
21 to 30 . . . .	3 "	53.4	1 "	78.6	4 "	59.7
31 to 40 . . . .	8 "	167.6	0 "	0	8 "	167.6
41 to 50 . . . .	11 "	233.4	1 "	143.0	12 "	226.1
51 to 60 . . . .	10 "	590.8	4 "	610.6	14 "	524.9
61 to 70 . . . .	10 "	552.8	3 "	300.0	13 "	494.3
71 to 80 . . . .	1 "	998.0	2 "	710.3	3 "	806.2
	52 cases		11 cases		63 cases	

Since 4 aortas were analyzed in both groups, Table III gives the results for Series I, as previously reported, and adds 11 new aortas in Series II.

The gradual increase in calcium by decades found in Series I was verified in Series II and compared in all respects.

When we attempted to determine the average calcium content according to types of lesions as we had outlined them for Series I, we were unable to find clear-cut examples of some of the types in Series II. This was due to the ways in which the types were determined. In Series I the basis of classification was largely microscopic, while in Series II both microscopic and gross changes, as they affected an entire segment, were thoroughly considered. Sharply defined type lesions limited to a single aortic coat were found in early arteriosclerosis but in the more advanced cases types overlapped and the

TABLE IV

*Relative Amounts of Calcium Deposited According to Type of Arteriosclerosis*

	Type of lesion	No. of segments	No. positive for calcium micro-chemically	Average calcium by analysis
				mg.
(a)	Negative.....	1	0	48.0
(b)	Intimal fatty streaks.....	6	0	80.0
(c)	Atheromatous cysts.....	7	4	229.0
(d)	Atheromatous cysts with medial calcification.....	7	7	319.7
(e)	Atheromatous cysts with calcified plates....	7	7	611.0
(f)	Large calcified plates in one or both layers..	6	6	1294.0
(g)	Aortitis with medial degeneration.....	4	0	224.5
(h)	Aortitis and atheromatous cysts.....	4	1	187.0
(i)	Aortitis and calcified plates.....	2	2	561.0
		44*	27	

\* Based on 15 aortas with three segments each. One segment was lost through premature opening of the autoclave.

processes commonly involved both intimal and medial layers. The latter condition applied to the calcium plaques as well as to other forms of the sclerotic process. Only those type lesions actually found in Series II are included in Table IV. The quantities given are relative at best, and are cited as indicating trends in calcium content with reference to type lesions and in no way as limiting amounts. Several of the segments shown in Table IV were borderline examples that might have been interchanged between groups (d) and (e) or between groups (e) and (f). Such readjustments could have been made without seriously upsetting the classification or the average calcium content for the particular group concerned.

The next step should have been to make a complete table showing the relative amounts of calcium found in each type of lesion for

the entire 63 aortas, but owing to the difference in the ways in which the lesions in the two series were typed, we had no common basis for such a comparison. Moreover, there were 20 cases under 40 years of age in the first series and only 2 under 40 in the second. To put the two series on a comparable basis, an age correction by decade would have been necessary. With this explanation we are presenting the figures just as we obtained them.

#### *Fatty Streaking of the Intima*

Series I — 10 segments averaged 77.2 mg. of calcium.

Series II — 6 segments averaged 80 mg. of calcium.

#### *Nodular Intimal Thickening*

Series I — 10 segments averaged 168 mg.

Series II — The lesion was not found in a segment where other more advanced lesions were not also present.

#### *Atheromatous Cysts*

Series I — 15 segments averaged 298 mg.

Series II — 7 segments averaged 229 mg.

Grumous and crystalline cysts appeared to be stages in the same process and were commonly found in the same segment. The crystalline cyst appeared to be the more advanced lesion and to contain slightly greater amounts of calcium.

#### *Diffuse Medial Calcification*

Series I — 13 segments averaged 345 mg.

Series II — All instances of medial calcification were complicated by atheromatous cysts. Seven segments which contained cysts and medial calcification averaged 319.7 mg. This suggested that cysts were present in the first series and were overlooked.

#### *Medial Cysts*

Series I — 8 segments averaged 770 mg.

Series II — Medial cysts were always found associated with other lesions.

#### *Calcified Plates (rarely were found limited to either layer)*

Series I — 13 segments averaged 1009 mg.

Series II — 6 segments averaged 1294 mg.

From this summary it may be seen that there is, in general, a comparative relation that applies between the type of lesion and the quantity of calcium recovered analytically, in spite of individual variations.

### AORTITIS

In the combined series there were 17 aortas where aortitis was an outstanding feature. Three of the cases showed exudation in the intima and media and were associated with general septicemia and septic processes due to some form of streptococcus. The calcium content of the 3 cases averaged 228 mg. One instance was typical

TABLE V  
*Calcium Content in 11 Cases of Luetic Aortitis*

Case No.	Age	Sex	Color	Arch	Thoracic	Ab-dominal	Average	Diagnosis
A-29-86	yrs. 38	F	W	mg. 90.0	mg. 853.0	mg. 131.0	mg. 358.0	Atheromatous cysts and aortitis
A-30-25	38	M	W	..	258.0	..	258.0	Nodular intimal aortitis
A-29-96	42	M	B	330.0	413.0	380.0	374.3	Nodular thickening and aortitis
A-34-8	49	M	W	309.0	72.0	48.0	143.0	Atheroma and aortitis
A-29-76	50+	M	W	114.0	121.0	226.0	153.6	Atheroma and aortitis
A-30-32	50+	M	W	111.0	130.0	957.0	399.3	Calcified plates and aortitis
A-29-78	54	M	W	182.0	213.0	923.0	439.3	Calcified plates and aortitis
A-30-5	58	M	W	475.0	355.0	418.0	416.0	Thickening and aortitis
S-34-190	61	M	W	288.0	200.0	512.0	333.3	Calcified plates and aortitis
A-34-39	64	F	W	Lost	161.0	610.0	..	Aortitis
A-34-28	75	M	B	220.0	178.0	191.0	196.3	

of the condition described in rheumatoid arthritis and occurred in an arthritic patient 48 years of age (A-30-14). The calcium was uniformly distributed in the three segments and averaged 153 mg.

The remaining 13 cases were from luetic patients. One was from an infant 5 months old. Another was complicated by secondary sarcomatous infiltration of the adventitia. The rest are shown in Table V.

Several facts are brought out by Table V. The distribution of the calcium was so uneven that the average content failed to have

any value and the amount of calcium recovered appeared to depend on associated arteriosclerotic processes rather than on aortitis. In segments where aortitis was present as the predominant condition the calcium was constantly lower than expected. In the majority the only calcium brought out by staining was shown by alizarin in the form of bright red granules on the remaining elastic fibrils.

### CONCLUSIONS

From the careful examination of 15 aortas selected according to the type of lesion present and in which detailed gross, microscopic and chemical calcium data were compared, we reached the following conclusions:

(1) There is a consistent increase in the amount of calcium in the aorta as the years advance, which is independent of the "type lesion" present.

(2) There is in addition an increase in the amount of calcium which depends upon the amount of arteriosclerosis present in the individual case and which varies with the kind and number of type lesions.

(3) The type lesions of arteriosclerosis may be classified separately for study but each represents a step in a progressive disease and, as such, is rarely encountered singly in any given sclerotic aorta.

(4) The increase in calcium appears to depend on two processes: that associated with elastin in the media, and that depending upon lipoidal degenerations in the contents of atheromatous cysts. The processes may operate singly but as a rule they go on simultaneously.

(5) The highest calcium values were obtained from the calcium plates that appeared to be relatively stable end products. Such plates were most commonly found in the abdominal segments.

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# STUDIES OF A PARALYSIS SYNDROME PRODUCED IN RABBITS AND GUINEA PIGS BY EXTRACTS OF NORMAL PRIMATE BONE MARROW \*

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In 1933 Friedemann and Elkeles<sup>1</sup> reported that in connection with studies concerning the etiology of pernicious anemia and acute leukemia they had observed that the intrathecal injection of bone marrow material into rabbits resulted in a very striking syndrome. These investigators further observed that normal human bone marrow when injected intrathecally into rabbits gave results identical with those obtained with marrow from their pernicious anemia and leukemia cases.

The symptoms produced in rabbits, as reported by Friedemann and Elkeles, and as we have noted them, develop after an incubation period of from 3 to 8 days — in a few instances after a somewhat longer period. Usually the first evidence of disease is spasticity with slight impairment of function of the hind legs. This paralysis may gradually progress and become more extensive without intermittence, and the animal may die of a generalized paralysis; or there may be a temporary arrest of the process, with relapse a few days later and then a fatal termination. The course of the affection is usually somewhat protracted, ordinarily extending over a period of from 2 to 5 weeks. Friedemann,<sup>2</sup> in a more recent article, states that when the inoculum is administered intracerebrally, as in all of our work, rigidity is characteristic, whereas a flaccid type of paralysis follows intrathecal injection of the bone marrow.

In our experiments we have produced the condition in guinea pigs and find that it is much more acute in such animals than in rabbits. Guinea pigs, at the onset of symptoms, frequently show evidence of marked excitability, a condition not noted in rabbits. This is followed by a progressive weakness and paresis of the hind quarters which rapidly becomes generalized and the animal usually dies within 4 to 8 days.

In their original report Friedemann and Elkeles called attention to the similarity of the disease they produced in rabbits with bone marrow with that observed by Gordon<sup>3</sup> when he injected intra-

\* Received for publication August 17, 1935.

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cerebrally into rabbits suspension of gland tissue from cases of Hodgkin's disease. Gordon believed he was dealing with a virus of etiological importance in lymphadenoma. In his second report Friedemann also draws an analogy between the bone marrow agent and the proteolytic ferment of Jochmann.<sup>4</sup> He also found the active agent in normal human spleen and leukocytes—a finding that coincided with the distribution of the Jochmann ferment.

Although in his early work Friedemann reported transmission of the affection, he was not subsequently able to repeat the finding and his later conclusion was that the agent was not a virus.

The experiments reported herein had several objectives.

In the first place we deemed it desirable further to investigate the possibility that the active agent was a virus.

If the active principle did not prove to be a virus, another possibility was that the bone marrow agent might be injected simultaneously with a virus not ordinarily pathogenic for the species of animal used, and pave the way for the establishment of the particular virus disease.

A further question to be settled was whether or not homologous bone marrow extract would produce disease. It was also desirable to see if homologous serum injected with an active bone marrow extract would prevent development of the disease.

Study of the histopathological changes in the brain tissue in this phenomenon seemed desirable. While the disease in rabbits had been referred to by Friedemann as an encephalitis, there was no evidence to indicate whether or not microscopic changes in the brain, commonly considered typical of encephalitis, occurred in the bone marrow-produced disease. As our studies advanced we were convinced that the disease produced by the bone marrow extract was not due to a virus but to a substance undoubtedly derived from the leukocytes. Thus, it was thought that a study of the histopathological changes in the brain might possibly throw light on the precise mechanism of brain tissue injury in encephalitis of known virus origin and in cases of postvaccinal encephalitis, and so on.

We have pointed out that Friedemann considered the possibility that the agent was identical with the proteolytic ferment of Jochmann which had been shown to occur in the bone marrow, spleen, and leukocytes of man and monkeys, and, to a lesser degree, in the same tissues of dogs. In our investigations we have confirmed the

presence of the paralysis-producing agent in the bone marrow and spleen of man, the bone marrow and leukocytes of monkeys, and in involved lymph nodes in Hodgkin's disease in man. We did no work with leukocytes from man or spleens from monkeys. One very potent extract was obtained from the spleen in a case of leukemia in man. We failed to obtain the active principle from horse, rabbit, and guinea pig bone marrow and spleen.

In general, extracts prepared from normal human spleens were of low potency and in many cases failed to produce disease. Bone marrow extract invariably proved potent, and that made from material taken from monkeys was more active than that of human origin.

In preparing our first extracts of spleen tissue we followed the method described by Jochmann and Lockemann<sup>5</sup> for the extraction of the "Jochmann ferment." This method is briefly as follows:

The tissue to be extracted is well broken up in a mortar, placed in a sterile Erlenmeyer flask, rubber-stoppered, and incubated in a water-bath at 55° C. for 24 to 48 hours. This results in a considerable amount of autolysis. Five volumes of a 2 to 1 absolute alcohol and ether mixture are then added, the mixture shaken, and allowed to remain at room temperature for 24 hours. The supernatant fluid is then drawn off and discarded and the residue dried in a desiccator. As soon as dry it is mixed with an equal volume of a 50 per cent solution of glycerine in physiological saline solution, is well shaken, and permitted to remain at room temperature for 24 hours. The undissolved material is removed by centrifugalization and the supernatant fluid carefully drawn off and saved, the precipitate being discarded. Five volumes of the alcohol-ether mixture are then added to the glycerine-saline extract. A flocculent precipitate results which usually settles rather rapidly. After settling of the precipitate, the supernatant fluid is drawn off down to the precipitate zone and discarded. The lower stratum of fluid containing the precipitate is centrifugalized and the supernatant fluid thoroughly drained off. The precipitate contains the active agent and can be dissolved in varying quantities of physiological saline solution up to twice the original volume of tissue.

As indicated, normal spleen extracts varied in potency. Hence, after our initial work we carried out all our experiments with bone marrow extract except when material from cases of Hodgkin's disease or leukemia became available, when we then used involved lymph nodes and spleen and obtained highly potent extracts. Further, in making bone marrow extracts we obtained satisfactory results with a simple method of extraction described by Friedemann.<sup>6</sup>

The bone marrow is placed in a sterile beaker and subjected to the action of several volumes of acetone for 15 minutes. The mixture is then poured on a

filter paper in a funnel and the acetone filtered off. The residue is washed with absolute alcohol and this followed with ether. After the ether has filtered off the filter paper can be spread open and the residue will dry rapidly. This dry powder is then taken up in an equal volume of 25 to 50 per cent glycerine in physiological saline solution, is well shaken, and left at room temperature for 24 hours. At the end of such period any undissolved material is removed by centrifugalization and the supernatant fluid carefully removed and saved. The glycerine-saline solution is then diluted with 5 volumes of a 2 to 1 absolute alcohol and ether mixture, this resulting in a flocculent precipitate which rapidly settles out. When the precipitate has settled (usually within half an hour), the supernatant fluid is siphoned off and the precipitate collected by centrifugalization. This results in a packing of the precipitate and makes it possible to turn the tube upside down and drain off the remaining alcohol-ether. If a concentrated extract is to be employed it is well to remove all traces of alcohol and ether through the use of washed air or vacuum. The precipitate is taken up in several volumes or more of saline solution and is ready for testing.

The potency of all of our extracts proved of very short duration, the final extract, at room temperature, ordinarily losing much of its activity in 24 hours and becoming completely inert within 48 hours. When stored in the refrigerator, loss of potency, strange to say, is even more rapid, the material often proving inactive after several hours. Friedemann explains this on the grounds that the active principle is caught up in fatty material which coagulates at ice-box temperature, and states that if these clumps are ground up in a mortar and injected the typical paralysis is produced.

Aside from the material from cases of Hodgkin's disease and leukemia, our bone marrow and spleen tissues were obtained at early autopsies. Our monkey bone marrow was obtained from normal *Macacus rhesus* monkeys, sacrificed for the purpose.

In all our experiments injections were made intracerebrally under ether anesthesia. With but few exceptions the dosage employed in all of our work was 0.3 cc. for rabbits and ferrets, 0.2 cc. for guinea pigs, and 0.05 cc. for mice.

We tested a number of our extracts for proteolytic activity. This was accomplished by placing a small amount of the extract in a tube of Loeffler's coagulated serum medium, allowing the extract to extend about half way up the slant. After 24 hours at room temperature the tubes were examined for evidence of proteolytic action by the extract. Extracts prepared from spleen tissue often proved non-proteolytic and were inert when inoculated into rabbits. Bone marrow extract invariably proved proteolytic.

In our studies to determine the possible presence of a virus as the

active principle of the extracts, numerous rabbits were injected intracerebrally with fresh extracts, then sacrificed at various intervals following inoculation, ranging from 24 hours subsequent to inoculation — and before symptoms developed — up to several weeks when the animals were in the terminal stages of the paralytic disease. Emulsions of the brain tissue of such animals were immediately inoculated into normal rabbits. In a number of these experiments, in removing brain tissue from the initially inoculated animal for transmission tests the tissue was carefully obtained from the area around the needle track with the thought that if we were dealing with a virus it might be localized at such a site.

Extracts prepared from normal spleen, leukemic spleen, glands from cases of Hodgkin's disease, and bone marrow were used in these transmission studies. In not a single instance were we able to transmit the disease from an affected animal to one that was normal.

In a further attempt to build up evidence for or against the bone marrow principle as a virus, brain tissues containing known neurotropic viruses were subjected to the same extraction processes as used in our experiments, and then inoculated into animals to determine whether or not the viruses would survive such treatment. For this phase of our experiments the viruses of St. Louis encephalitis, equine encephalomyelitis and rabies were used. In all instances the extraction process rendered the viruses inert.

To determine whether or not damage of the brain tissue by bone marrow extract would make it possible for a virus to propagate in a species of animal for which it was not ordinarily pathogenic, we carried out several experiments.

In one series the virus of St. Louis encephalitis, which does not produce disease in guinea pigs, was mixed with bone marrow extract and the mixture injected into guinea pigs and mice. The guinea pigs developed the typical paralysis due to the bone marrow extract, but when portions of their brains were removed, emulsified and injected into additional guinea pigs and mice all such animals remained healthy. It was evident, therefore, that the St. Louis encephalitis had not survived in the original guinea pigs receiving the mixture of virus and bone marrow extract. The mice — normally susceptible to St. Louis encephalitis — developed the St. Louis virus disease after the usual incubation period. Control mice, receiving only the bone marrow extract, remained well.

During the period these experiments were in progress we received, through the courtesy of Dr. Maxwell MacDonald, a number of specimens of spinal fluid from cases of encephalitis of undetermined etiology. We have been routinely inoculating such specimens into mice, guinea pigs, rabbits, and occasionally ferrets, with the hope of picking up a virus. In two instances such specimens were received when we happened to have on hand freshly prepared bone marrow extract. Under such circumstances we inoculated mice, guinea pigs, rabbits, and in one instance ferrets, with mixtures of the spinal fluid specimens and bone marrow extract. We failed to recover a virus through the adjunct use of the bone marrow extract.

In another case of fatal encephalitis from which brain material was received through the courtesy of Dr. William Holt, we employed bone marrow extract in inoculation tests in attempts to increase the pathogenicity of a suspected virus. In this case — even without the bone marrow extract — we obtained fairly definite evidence of the presence of a virus. However, after passage through three series of half grown mice it was lost. The pathogenicity of this "virus" for mice was not enhanced through the adjunct use of bone marrow extract.

An interesting question in connection with these studies was whether or not these paralysis-producing extracts would cause the disease in the species from which tissues were obtained for the preparation of extracts. Accordingly, we inoculated monkeys intracerebrally with a highly potent monkey bone marrow extract. The monkeys developed no evidence whatever of the disease, whereas guinea pigs and rabbits inoculated with the same material developed the classical paralysis and succumbed.

One experiment was conducted to determine whether or not fresh, normal monkey serum would neutralize the active principle in monkey bone marrow extract. For this purpose two rabbits were each injected intracerebrally with 0.4 cc. of a mixture of equal parts of the bone marrow extract and monkey serum, the mixture being allowed to stand at incubator temperature for 30 minutes before injection. Similarly, two control rabbits were each injected with mixtures of the bone marrow extract and fresh, normal rabbit serum. All four rabbits developed the typical paralysis, indicating that neither the homologous serum nor the rabbit serum possessed ability to neutralize the active principle in the bone marrow extract.

## HISTOLOGICAL STUDY

For purposes of histological study, six guinea pigs, with intracerebral injections of both human and monkey bone marrow extract, were subjected to careful examination. These animals were allowed to live until their behavior indicated that they probably would not survive until the next morning. This interval ranged in different animals from 4 to 9 days following injection. They were then killed with chloroform. Four of these animals were perfused through the heart with 10 per cent formalin, the brains embedded in celloidin, sectioned serially at 20 microns, and every tenth section stained with thionin. In two instances the brains were removed and fixed in formalin without preliminary perfusion. These were embedded in paraffin, sectioned serially at 10 microns, and every fifteenth section stained with thionin. Portions of the latter material were cut at 5 microns to allow more detailed cytological observation. In addition, sections from the brains of a large number of similarly inoculated guinea pigs were studied, but as these were not serial sections they were used only for purposes of confirmation.

Considerable rabbit material was also available, but for careful study the guinea pig was chosen in preference to the rabbit, since in the latter spontaneous encephalitis is much more likely to be a complicating factor. This factor will be discussed subsequently.

For control purposes three normal stock guinea pigs were killed with chloroform, perfused with formalin, the brains embedded in celloidin, cut serially, and every tenth section stained with thionin. For further controls the brains of two guinea pigs injected intracerebrally with non-pathogenic rabbit bone marrow extract were studied in serial sections. These animals received 0.3 cc. of rabbit bone marrow extract, prepared in exactly the same way as was the human or monkey material. Five days following injection they, too, were killed with chloroform, perfused through the heart with formalin, the brains embedded in paraffin, and every fifteenth section, at 10 microns, stained with thionin.

The pathological findings fall naturally into two groups: the local action at the site of injection, and the more general, widespread effects, which we call action at a distance.

The lesions at the site of the injection are a familiar picture, rather similar to that of any brain puncture accompanied by the injection of a small quantity of readily absorbed material. The typical histological appearance is indicated in Figure 2 (G. P. No. 8). There is a central zone of necrosis, with a moderate amount of hemorrhage and vast numbers of Gitter cells. Occasional leukocytes may be



seen, but their presence is not marked. More peripherally there is a zone of edema and cytological change, but without necrosis. In this reactive zone there is marked increase in the number of glial cells, chiefly the microglia, which show marked progressive alterations. In addition there is present a moderate amount of cellular débris and nuclear fragments, representing phagocytes degenerating *in situ*. Gitter cells are prominent, intermingled with the transitional forms of microglia. There is a moderate amount of vascular proliferation, with a slight degree of adventitial thickening. Around some of the blood vessels there is a very minor increase in perivascular cells which appear to be chiefly mobile glial cells.

The neurons within the reactive zone may show swelling and chromatolysis. Such changes are not, however, a necessary part of the picture, since nerve cells morphologically completely normal may exist in the midst of marked glial alterations.

In Figure 1 the site of injection was superficial in the hemisphere. There is a dense collection of cells, chiefly microglia, on the extreme left, while in the adjacent cortex a diffuse loss of neurones in the outer cortical laminae is readily apparent. Coincidental with the loss and injury of nerve cells is a marked glial reaction coupled with a proliferation of blood vessels. All of the neurones are not destroyed; many remain in various stages of degeneration. Examination of Figure 1 shows, moreover, a loss of neurones, with proliferation of glia and of blood vessels, in the dorsomedial cortex of the opposite hemisphere. This picture suggests that the pathogenic agent extended over the surface of the brain in the subarachnoid space. In this particular case the destructive process, of the type illustrated, involved the superficial layers of the cortex over approximately one-half of one entire hemisphere, with a slight involvement of the opposite hemisphere.

Lesions of the type illustrated here might be caused by the application of any of numerous destructive agents. The type of destruction observable in Figure 1 might equally well be produced by the local application of heat, or the injection of alcohol into the brain and subarachnoid space.

The meninges of the animals receiving the primate bone marrow extract all showed abnormality. The reaction, however, was not necessarily at the site of the inoculation. In Figure 1, for example, the meninges covering the reactive cortex are not especially abnor-

mal. This animal, however, had a moderate degree of basal meningitis, not illustrated here. In other cases, on the other hand, there is a pronounced inflammation between hemisphere and brain stem. Yet the infiltration is even more pronounced at the base of the brain, and between the opposite hemisphere and brain stem. In general, the meninges over the convexity of the brain are quite normal, while inflammation is present at the base of the brain and between the hemispheres and the brain stem. This inflammation is non-purulent. Although polymorphonuclear leukocytes are present, mononuclear cells predominate.

Apart from the focal lesions there are numerous interesting changes which we call action at a distance. The meningeal reaction, independent of the site of the injection, is such a case. By far the most striking reaction is the involvement of the cerebellum, present in every single animal examined, including the six brains sectioned serially, and about ten other guinea pigs and rabbits whose brains were studied histologically, though not in serial section.

All of these animals exhibit an identical picture. The Purkinje cells are most severely affected. A low power view of two typical folia is shown in Figure 3, while a perfectly normal section with the same fixation and staining and of the same thickness is shown below. In Figure 3 the loss of Purkinje cells is obvious. In the lower folium, and in the lower part of the upper folium, not a single Purkinje cell remains. In their place is seen a row of cavities and small cysts, with a moderate glial reaction. In the uppermost part of Figure 3 a layer of pale staining Purkinje cells is apparent. Under higher power it is clear, not only that the staining reaction of these cells is much weaker than normal, but that they show very severe morphological alterations.

Degenerative changes in the Purkinje cells are rather varied. The simplest and least malignant form affects only the cytoplasm, which becomes swollen and sometimes vacuolated, with a dissolution of chromatin material, especially in the periphery of the cell, accompanied by a general pallor of staining reaction. The nucleus, although it may be displaced toward the periphery of the cell, remains essentially intact.

In more severe changes the nucleus is affected. The nuclear membrane shrinks, becomes irregular, or may disappear. The basophil nucleoli become fragmented, separate from the true nucleolus, and

become dispersed throughout the nucleus. Where the nuclear membrane undergoes dissolution the fragments of basophil nucleoli, with their characteristic blue-green staining reaction, may become dispersed through the cytoplasm. In other cases the entire nucleolar complex may be displaced, through a ruptured nuclear membrane, into the cytoplasm.

Sometimes the cytoplasm is shrunken, homogeneous and very pale, while the entire nucleus stains a very deep blue. In still other cases the entire cell, cytoplasm as well as nucleus, is shrunken and exhibits a uniform intense blue staining.

As the Purkinje cells degenerate and disappear there is a glial reaction leading to the formation of the glial "Strauchwerk" described by Spielmeyer.<sup>7</sup> This affects chiefly the microglia of the molecular layer and the Bergmann glia. This characteristic reaction is most recently described by Scherer<sup>8</sup> who gives a full bibliography.

Apart from the changes in the cerebellar cortex, there are two other types of action at a distance which deserve mention, affecting, respectively, the neurones and the microglia.

In all cases there are widely scattered through the brain stem groups of neurones showing the primary irritation of Nissl,<sup>7</sup> *i.e.* swelling, chromatolysis and eccentric displacement of the nucleus. With the exception of the large celled, anterodorsal nucleus of the thalamus, which was affected in all cases, there are no single nuclear configurations invariably damaged. Nevertheless, every case showed extensive changes in widely scattered cell groups over the entire brain stem. The spinal cord was also affected. Figure 5 of Deiters's nucleus, in the same animal from which Figure 2 was taken, is a typical field, although by no means as pronounced as may be sometimes found.

Curiously, the motor trigeminal nucleus is spared in all cases. The cell groups most frequently affected, apart from the anterodorsal nucleus of the thalamus, are the vagus, hypoglossal and vestibular nuclei, the inferior olivary complex and the pontine nuclei, together with scattered large cells in reticular formation throughout the neuraxis. Other cell groups may less frequently be affected, with the one exception noted above.

A further change from the normal consists in the swelling of the microglia, of the type described by Alpers<sup>9</sup> in urea poisoning, and

subsequently observed in other conditions. Proliferation of the microglia is frequently present in addition.

It is to be emphasized that in perfectly normal guinea pigs the microglia, seen in thionin preparations, sometimes show a well outlined cell body with thickened retracted processes identical with the changes described by Alpers on the basis of silver preparations. The presence of isolated cells of this type is not a sign of abnormality. But in our experimental material there is considerable increase in the number of such cells, which we feel to be of significance in the pathology of this condition. The occurrence of this change is especially to be noted in the midbrain, pons and hypothalamus. This behavior of the microglia is usually, but not always, to be observed where the ganglion cells show primary irritation. On the other hand, the glial changes may be present even where the neurones are unaffected.

The blood vessels apart from the vicinity of the injection site usually show no change. Proliferation of blood vessels, with thickening of their adventitia, and slight accumulation of mononuclear cells therein, may rarely be observed in parts of the brain showing no contiguity to the original lesion. Occasionally, in the rabbit material examined, small discrete areas of softening, together with marked hematogenic infiltration of perivascular spaces, are present far removed from the lesion. Uncertainty whether this was a result of the lesion, or a coincidental spontaneous encephalitis, led us to depend on the guinea pig. In our series of the latter animals we have never observed any changes that could possibly be confused with a spontaneous encephalitis.

In all our material the cerebral cortex, apart from the point of entry of the needle, or the reaction shown in Figure 1, is entirely normal. Many cells show what are apparently severe alterations, especially shrinkage and deep staining reaction of both cytoplasm and nucleus. The pyramidal cells of the third and fifth layers particularly show this reaction, as well as many cells of the pyriform lobe and other parts of the olfactory cortex. On the other hand, cells of identical appearance are to be found in normal control material in the same cortical areas, and consequently cannot be considered abnormal.

## DISCUSSION

While there are some things in connection with these bone marrow and other tissue extracts, and the disease produced by the same, that suggest the possibility of the presence of a virus, there are a number of points in opposition to such theory.

The definite incubation period in all cases, and the occasional intermittent type of case, would naturally raise the question as to whether or not a living agent was involved in the production of the disease. Then, the similarity between the active principle in bone marrow, normal spleen and leukocyte extracts, and that in the lymphadenoma preparations which Gordon worked with and considered virus, emphasized the necessity of considering the virus possibility. On the other hand, the analogy between our extracts and the "Jochmann ferment" is in opposition to the virus theory. Further, it would have been very unusual indeed had we found a virus capable of withstanding the severe chemical treatment employed in the preparation of our extracts. Our negative transmission tests reported herein were not, therefore, unexpected.

Had it proved possible to activate, with bone marrow extract, some of the known neurotropic viruses, in animals not otherwise susceptible to it, the finding would have proved very useful and would have thrown considerable light on some phases of our virus problems. Our negative results with a known virus seem to indicate that the bone marrow extract is of no value for the purpose. This tends to indicate that such extracts are of no service in "bringing out" viruses that might possibly be present in material from some of the cases of encephalitis of undetermined etiology.

The outcome of our inoculation experiments with homologous extracts proved that there is no danger of an "auto-infection" with the paralysis-producing agent present in normal human bone marrow, spleen and leukocytes.

As regards the histopathology, there are two chief points on which discussion may be based, *i.e.* what is the explanation of the various pathological changes described above, and what is the relation of these morphological changes to the physical signs and clinical appearances of the injected animals?

The pathogenic bone marrow extract is known to have a proteolytic effect *in vitro*. The intensity of the local action at the site of in-

jection is undoubtedly in relation to this property. But in a qualitative sense the primate bone marrow has given us a picture similar to that which could be produced by any of numerous destructive agents.

It is clear, however, that the clinical picture resulting from the injection is not the result of the local destruction of brain tissue. As has been demonstrated in the accompanying photographs, the location and extent of the focal lesion vary quite widely from one animal to another. The clinical syndrome, if we may be allowed the term, shows a great constancy. Furthermore, the destruction produced by the non-pathogenic material did not cause the characteristic clinical picture, or any part of it.

Pathological changes away from the site of injection (what we have called action at a distance), namely, the severe alterations in the cerebellar cortex, the widespread primary irritation of ganglion cells and the "toxic" changes in the microglia, cannot be held responsible, *per se*, for the clinical picture. None of these changes is in any sense specific. There are over thirty conditions (Scherer<sup>8</sup>) in which more or less similar cerebellar pathology may be found, ranging from epilepsy to olivo-ponto-cerebellar atrophy to dysentery. So, too, ganglion cell and glial changes similar to those described above may be observed in a wide variety of clinical conditions.

It is our belief that no exact correlation can be made between the pathological findings and the symptomatology. Before death the animals gave evidence of a widespread involvement of the nervous system, analogous to that found in a virus encephalitis. The present condition is most certainly not due to a virus, nor can the term encephalitis be used without some violence to usage. Nevertheless, it is probable that the pathogenic agent has acted upon the nervous system as a whole. To this widespread action we would refer the symptoms.

It is equally probable that the morphological alterations visible under the microscope are due only to a special susceptibility of the regions affected to the noxious agent. Why one group of ganglion cells rather than another should show microscopic changes it is impossible to say. Why the neocortex, supposedly extremely sensitive to injury, should appear intact must remain unexplained for the present. The fact remains that scattered groups of cells, both neuronal and glial, give evidence of damage.

The cause of this damage is presumably the same as the cause of the symptoms. Yet, as mentioned above, it is unlikely that the symptoms as a whole are directly referable to the damaged cells visible under the microscope. We suggest that the fundamental pathogenic agent, whose exact nature and mode of spread must remain unknown for the present, acts on the nervous system as a whole. A functional disturbance is produced, leading eventually to death. Morphological changes are also produced in the course of this process. The data at hand are not adequate for further analysis.

### CONCLUSIONS

The paralysis-producing principle present in normal bone marrow, spleen and leukocytes of man and monkeys is not a virus or other type of living agent and is incapable of producing a transmissible disease.

This active, disease-producing agent is of a chemical or enzymatic nature, apparently of leukocytic origin, and is undoubtedly identical with Jochmann's proteolytic ferment and with what Gordon thought was a virus in Hodgkin's disease.

Extracts of high potency for rodents can be regularly prepared from bone marrow of man and monkeys, that from monkeys being especially active. Extracts prepared from normal spleen tissue are not nearly so satisfactory, a number of such preparations often proving inert. Lymph glands from cases of Hodgkin's disease, and spleen tissue from leukemia cases produce highly potent extracts. Extracts from leukocytes are apparently very active although we prepared but one (from a monkey) in our work.

Both rabbits and guinea pigs are readily susceptible to intracerebral injections of these extracts.

The simultaneous injection of bone marrow extract and a known neurotropic virus into a species of animal not ordinarily susceptible to the virus does not make possible the propagation of the virus in such species.

Bone marrow extract proved of no value in attempts to "bring out" a possible virus in cases of acute encephalitis of undetermined etiology.

Monkey bone marrow extract, injected intracerebrally into monkeys, does not produce disease, hence, it is evident that "auto-infection" does not occur.

Extracts prepared from bone marrow of the rabbit, guinea pig, and horse failed to produce disease when injected into rabbits and guinea pigs.

Homologous serum does not neutralize the active principle in bone marrow extracts.

The pathological findings are divisible into the local action at the site of injection and the action in other parts of the nervous system remote from the local injection, which we call action at a distance. The local action is similar to that resulting from the introduction of any destructive agent, but much more severe than would be expected from the small quantities of material injected. The action at a distance involves primarily the cerebellum, where there is severe and widespread injury to the Purkinje cells, with the formation of a glial "Strauchwerk." Other changes include pronounced but scattered primary irritation of ganglion cells, "toxic" swelling of microglia and meningeal infiltration, predominantly with mononuclear cells.

We believe that the symptoms of the animals are not the direct result of the lesions described. The symptoms seem to be the result of a widespread functional involvement of the nervous system, the exact nature of which remains unknown. The lesions at a distance are probably the result of a special vulnerability to the pathogenic agent.

#### ACKNOWLEDGMENTS

For the various specimens of human bone marrow and pathological tissues from cases of Hodgkin's disease and leukemia, we wish to acknowledge our indebtedness to Dr. R. E. Gross of the Peter Bent Brigham Hospital, Boston, Mass., Dr. Frederic Parker, Jr., of the Department of Pathology, Harvard Medical School, and Pathologist to the Boston City Hospital, Boston, and Drs. Sydney Farber and O. T. Bailey of the Children's Hospital, Boston, Mass.

Finally to Professor Hans Zinsser of the Department of Bacteriology, Harvard Medical School, under whose direction this work was done, we are greatly indebted for much help and advice.



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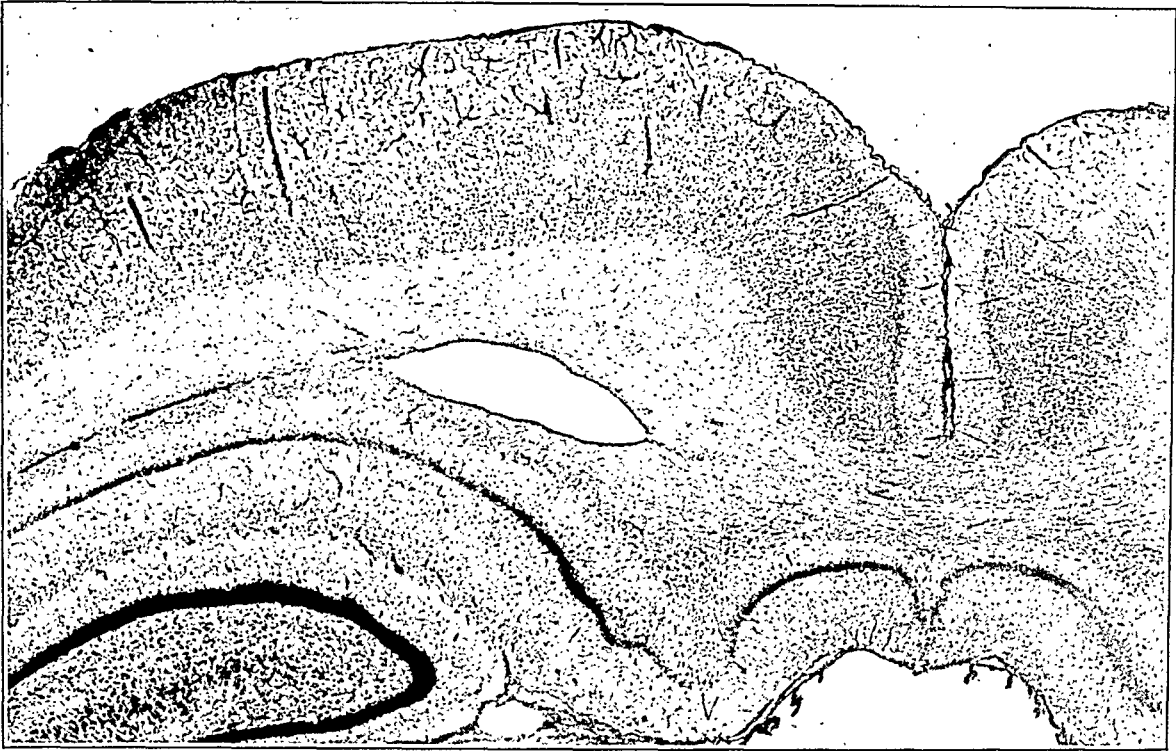
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DESCRIPTION OF PLATES

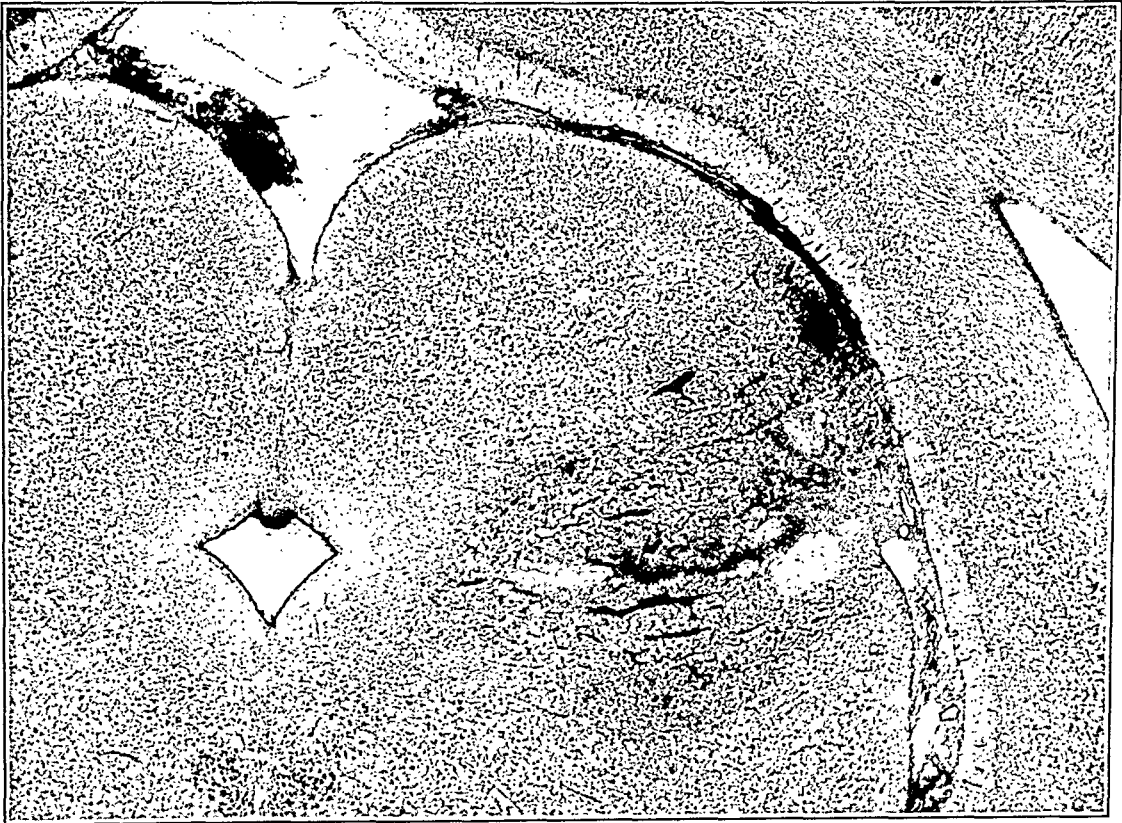
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## PLATE 36

- FIG. 1. Lesion when site of injection is superficial in the hemisphere (description in text).
- FIG. 2. Typical zone of necrosis, with hemorrhage (see text).



1

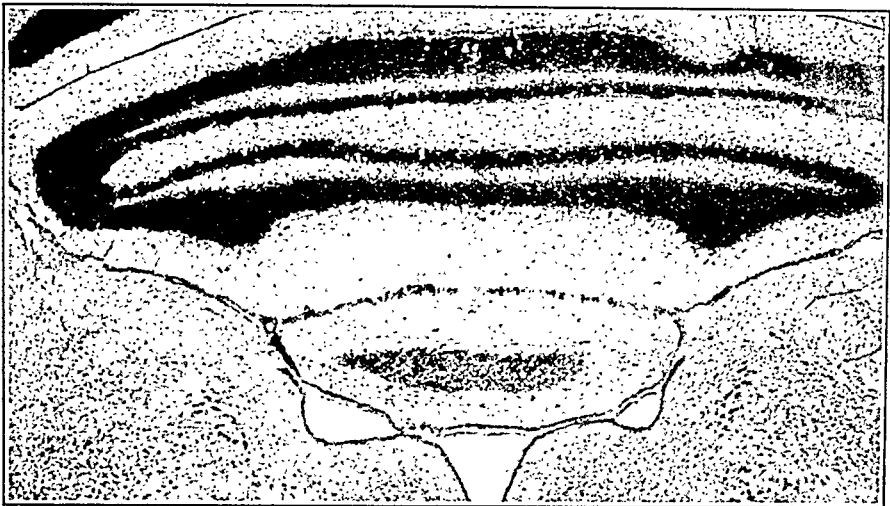


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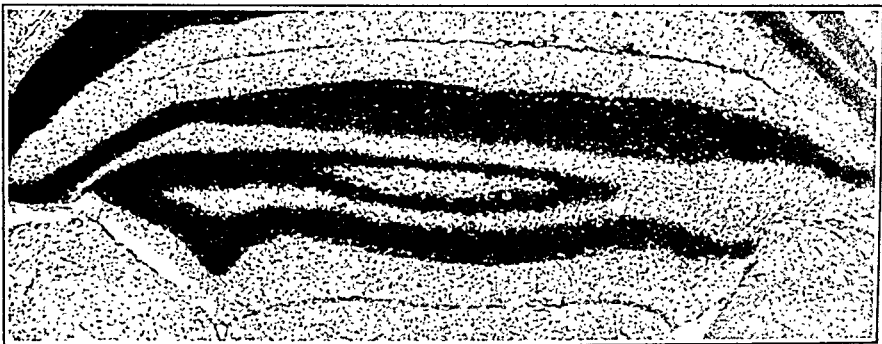
PLATE 37

FIGS. 3 and 4 contrast cerebellar lesions with normal cerebellum section, showing loss of Purkinje cells in Fig. 3 (see text).

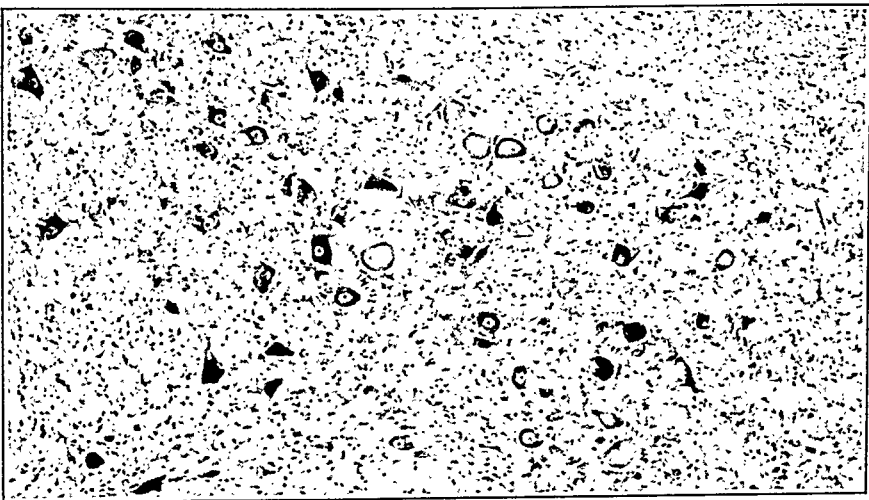
FIG. 5. Section of spinal cord (see text).



3



4



5



# THE CHANGES IN THE APPEARANCE OF THE WALL OF A MUSCULAR ARTERY BETWEEN DIASTOLIC AND SYSTOLIC BLOOD PRESSURES \*

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Sections of arteries removed postmortem do not represent the true appearance of the vessels during life. The lumens are seen to be small and bounded by a deeply folded intima and internal elastic lamina and the walls of the vessels are unduly thickened. It was suggested by Ballance and Edmunds,<sup>1</sup> and later proved by MacWilliam,<sup>2</sup> that this appearance is due to a postmortem contraction occurring in the musculature of the media which causes a great decrease in the diameter of the lumen and consequently throws the inner part of the wall of the vessel into numerous abnormal folds. The exact appearance of the wall of an artery distended during life by its normal blood pressure has not been universally agreed upon, nor is it known what changes take place in the wall during the passing of the normal pulse wave. It would seem that these conditions in normal arteries should be better understood to gain an appreciation of the appearances and effects of pathological lesions.

In the present investigation arteries were distended and fixed at their normal blood pressures and the appearance of the walls of these arteries was observed. It was hoped that the arteries would respond to distention under the experimental conditions imposed on them in a manner sufficiently similar to those *in vivo* so that the appearance of the wall, at diastolic and systolic blood pressures, and the changes which occur in the wall with the passing of the pulse wave, could be appreciated.

MacWilliam and Mackie<sup>3</sup> showed that the postmortem contraction which occurs shortly after death materially affects the histological appearance and physiological properties of arteries. Roy<sup>4</sup> had found that equal increments of internal pressure in arteries caused increasing augmentation of volume per unit rise of pressure, and

\* Received for publication October 3, 1935.

that arteries were most elastic and most distensible with internal pressures such as had existed during life. MacWilliam, however, found that with contracted arteries there was increasing augmentation of volume per unit rise of pressure up to a point of maximum distensibility and then diminishing increments. The point of maximum distensibility varied with the degree of postmortem contraction present in the musculature of the arteries. A strongly contracted artery required an internal pressure far in excess of normal blood pressure, whereas a weakly contracted artery required less than normal blood pressure to reach the point of maximum distensibility. Relaxed arteries behaved very differently, the increase in volume being greatest at the first rise of pressure above zero, and diminishing increments appearing with each rise in pressure thereafter. In other words, the contracted artery, after it has reached the point of maximum distensibility, behaves in a manner similar to that of the relaxed artery. Or again, the initial rise in pressure in a contracted artery is exerted to break the postmortem contraction present. It is obvious, therefore, that if one expects to observe the appearance of arteries under normal blood pressure they must be observed after this strong contraction has been removed. MacWilliam described several different methods by which this could be done: (a) freezing, (b) exposure to ammonia vapor, (c) heating, (d) kneading, rubbing, stretching, and so on, and (e) immersion in potassium sulphocyanide. Nakonetschny<sup>5</sup> also showed that the vessel could be relaxed by distending it with saline for a short time.

The last two methods mentioned were utilized in this experiment, and as more uniform results were obtained by immersing the arteries in potassium sulphocyanide, this was done in the majority of cases. In all, twenty-two different sets of arteries were examined. The arteries observed were the external iliac arteries from humans on whom a postmortem examination was made within 8 hours after death. When the arteries were relaxed with potassium sulphocyanide they were attached to a cannula and the branches ligated; the vessels were then filled with and immersed in an aqueous solution of this substance (20 gr. per 100 cc.) for 3 minutes and then washed thoroughly with saline. Formol-saline (10 per cent) at the required pressure was used to distend the vessels, which were then placed in formol-saline to fix, while still distended, for at least 24 hours. When the vessels were relaxed by a primary distention with saline,

an internal pressure of 80 to 100 cm. of saline was applied for a length of time varying from 5 to 10 minutes, and the final distention and fixation with formol-saline were the same as in the other method. In the first series the arteries from the opposite side were used as controls; later, both arteries were distended after segments were cut from each for controls. Sections were stained according to Verhoeff's method for staining elastic tissue.

It was observed that the degree of postmortem contraction in the control sections varied considerably. As other workers have pointed out, it was found that the number of folds and their depth in the internal elastic lamina is a criterion for the amount of contraction present in the muscle fibers of the media. Where the media is the thickest, the number of folds in the internal elastic lamina and in the intima is the greatest. It was found that the degree of contraction varied not only in different arteries but in different parts of the same segment of a single artery. Eleven cases were analyzed in an attempt to discover some cause for the differences in contraction. It was felt that the age, cause of death, blood pressure during life, and the number of hours after death that the arteries were examined, might be factors in determining the degree of contraction. No relation, however, was found between these factors and the degree of contraction in the arteries. Of the four factors, it was felt that the cause of death and the number of hours postmortem were the most important. There were many different causes of death in this series but the length of time after death that the autopsies were done ranged only from 1 to 8 hours. It is recognized that in excised arteries the extreme contraction present is due to a survival of excitability of the musculature and we believe the variation in stimulation caused by removing the vessels plays a large part in determining the degree of contraction present, and likely accounts for the differences in contraction seen in a section of an artery.

Twelve arteries were distended at a pressure equivalent to the normal systolic blood pressure. In these, results were obtained similar to those of Klotz,<sup>6</sup> Nakonetschny<sup>5</sup> and Wolff.<sup>7</sup> The folds of the intima and the internal elastic lamina became flattened out and the layers of the vessel wall took a circular course about the lumen. The media was thin, the elastic fibers in the media were long and straight and their branches difficult to discern. The fibers of the external elastic lamina were also straightened out. In some cases the



folds did not entirely disappear from the intima and the internal elastic lamina but gave way to long low rolls. In other isolated areas deeper folds were present and under these the media was found to be thicker than in the areas where the folds were lost. It was thought that these were areas that were strongly contracted, similar to the areas of increased contraction observed in the control vessels, and that the treatment used to cause a relaxation of the arteries had been insufficient for these parts.

Ten arteries were treated similarly and distended at a pressure equivalent to the normal diastolic blood pressure. In these it was expected that the waves in the intima and internal elastic lamina would be present in a moderate degree, but less than that seen in the contracted state. We expected to find a "position of rest" of the vessel wall such as Wolff described, in which he found a uniform waviness of the intima and internal elastic membrane in arteries distended at diastolic blood pressure. From his findings Wolff concluded that the pulsitory variation in the artery wall took place between this position of rest and a complete flattening of the folds. Contrary to expectation, however, it was observed that the appearance of a vessel wall distended at diastolic pressure by either of the methods used was identical with that of those distended at systolic pressures. Of the two arteries, in each of the last 8 cases examined, one was distended at the diastolic pressure and the other at either average or systolic pressure. Sections from the two distended arteries in each case were indistinguishable, one from the other, regardless of the method of relaxation and the pressure at which they were distended.

With few exceptions the arteries in these experiments were taken from individuals between 20 and 40 years of age, as it has been shown by Foster<sup>8</sup> that the elastic tissue, and by MacCordick<sup>9</sup> that the muscle tissue, is fully developed between these ages. Also, there are but slight arteriosclerotic changes at this age. Both by accident and by intention some arteries showing various degrees of sclerosis were included. In these it was observed that where there was only slight proliferation of the fibrous tissue of the intima, all parts of the wall showed a change after the distention. The intima and the internal elastic lamina lost their folded and wavy character. Where the sclerotic changes were marked, the layers of the vessel appeared to be fixed in a distended condition.

If it were assumed that the arteries *in vivo* respond to the changes in blood pressure in the same way as the arteries under these experimental conditions, it would be necessary to conclude that the vessel wall does not markedly change its appearance during the passing of the pulse wave. It is known, however, that there is a definite expansion of the vessel during systole which may be shown by pulse wave tracings. Hence, it must be concluded that excised arteries subject to the conditions present in this experiment do not give a vessel-wall curve similar to that of arteries *in vivo*.

It has been shown that the folds seen in contracted arteries tend to flatten out when the relaxed vessels are distended. The smooth muscle of the media is definitely able to contract and different degrees of contraction may be seen in untreated arteries. The greater the contraction of the muscle, the greater is the folding observed in the various layers. Dietrich<sup>10</sup> points out that there is a relaxation of the muscle in the media during the passing of the pulse wave, immediately followed by a contraction of the media after the wave passes by. Hence, it may be stated that there is a difference in the degree of folding present in the intima and in the elastic tissue at diastolic and at systolic blood pressures. If the normal tonus of an artery allows a complete relaxation of muscle, all the folds in these layers will disappear; but, on the other hand, long rolling folds may still be present. During diastole the waviness in these layers is undoubtedly less than that seen in excised arteries showing postmortem contraction of the media. Therefore, it is concluded that the pulsatory variation in the shape and appearance of the wall of arteries of the muscular type must be between a moderate degree of waviness in the intima and internal elastic membrane during diastole and a complete flattening of these folds during systole. We are not prepared, however, to state whether or not sufficient relaxation of the media occurs to cause an absolute loss of waviness of the intima and internal elastic membrane.

#### SUMMARY

The right and left external iliacs were removed *in toto* from 22 routine postmortem cases. One of the arteries of the first twelve pairs and both arteries of the remaining pairs were treated to relax the postmortem contraction of the media, and distended and fixed

at either systolic, diastolic or average blood pressures. Controls showing postmortem contraction of the media were kept, and of these, eleven were analyzed in an attempt to determine the cause of the differences in the degree of postmortem contraction observed. The experiment dealt primarily with normal, fully developed arteries, but some observations were made on arteries showing arteriosclerotic changes.

### CONCLUSIONS

1. The number of folds present in the intima and internal elastic lamina of muscular arteries is a criterion for the degree of contraction present in the muscle tissue of the media.
2. The degree of postmortem contraction in excised arteries is not uniform. It varies not only in different arteries from the same individual but in different parts of the same segment of an artery. These differences are due largely to differences in stimuli produced in excising the arteries, although other factors also probably play some part.
3. Relaxed excised arteries, distended by pressures equivalent to at least 80 mm. Hg. or more, show a loss of waviness of the intima and of the elastic tissues in the wall. The elastic fibers in the media and in the external elastic lamina lose their wavy contour before the fibers in the internal elastic lamina.
4. The changes in the artery wall during the passing of the pulse wave probably vary between a moderate folding of the intima and internal elastic lamina at diastolic blood pressure, and a lesser degree of this folding at systolic pressure, a change that may even extend to a complete loss of folds in these layers.

I wish to take this opportunity to thank Professor Oskar Klotz for his helpful suggestions and kindly criticism in this work.

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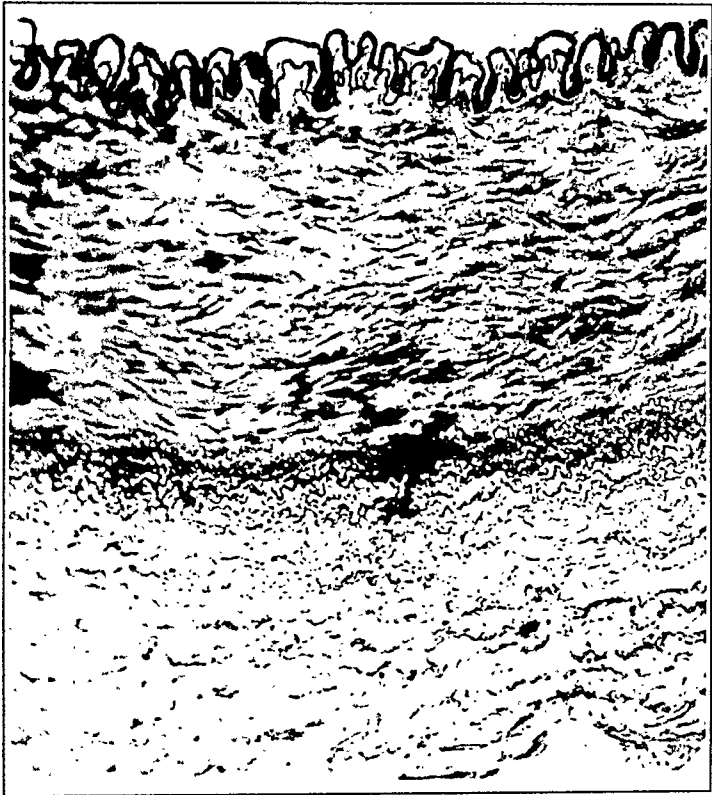
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## DESCRIPTION OF PLATE

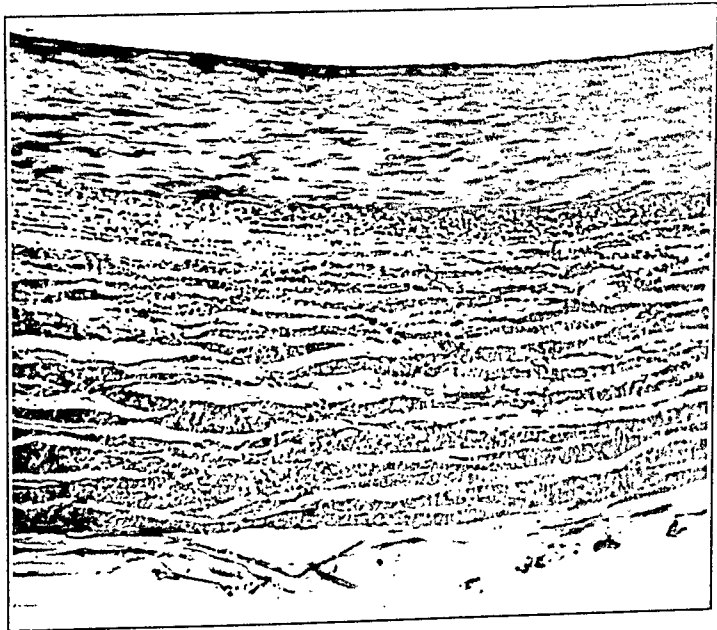
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### PLATE 38

- FIG. 1. Artery showing marked degree of postmortem contraction.  $\times 200+$ .
- FIG. 2. Artery relaxed by immersion in potassium sulphocyanide and distended at systolic pressure.  $\times 200+$ .



I



2



# CLINICAL AND PATHOLOGICAL FEATURES OF AN INFECTION CAUSED BY A NEW PATHOGEN OF THE GENUS *LISTERELLA* \*

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A new species of organism belonging to the genus *Listerella* was first obtained from a newborn infant in February, 1933. One year later a similar organism was recovered from the blood in 2 separate cases of fatal illness in infants, and soon thereafter a fourth organism of the same type was isolated at autopsy from the meninges and viscera of an adult. A brief clinical, epidemiological and pathological presentation of the findings in these 4 cases follows.

## CASE REPORTS

CASE 1. A-2810. The patient, a white male infant, was born at home at 1.00 A.M. on Feb. 17, 1933. It was the seventh child of apparently healthy parents. All the other children were living and well. It was said, however, that the mother had had a cold several days before delivery, but she had apparently recovered at the time of the birth of this child. Labor lasted for 20 hours, but it was a normal spontaneous delivery, occurring 2 hours after the rupture of the membranes. At birth the baby was cyanotic and did not cry immediately but had to be stimulated to breathe. Difficulty in breathing recurred later in the day and was accompanied by cyanosis, with the result that the physician sent the child to the hospital.

On admission the patient was moribund, breathing irregularly, gasping for air, and appeared cyanotic. The temperature was 101° F. The anterior fontanelle was open and not bulging. There was no evidence of injury to the head. Jaundice was not present. Heart sounds were poor in quality. There were numerous, fine crepitant râles heard over both lungs. Blood and lung cultures yielded a hemolytic Gram-positive bacillus.

The infant did not react to stimulation and expired within the hour.

## *Pathological Findings*

Postmortem examination performed 12 hours after death presented the following significant findings.

The body was well developed, weighing 3175 gm., and was 53 cm. in length. The only gross evidence of change observed was the

\* Received for publication October 10, 1935.



presence of atelectasis involving all of both lungs except the anterior margins.

Histologically the walls of the larger umbilical vessels showed some infiltration with small lymphocytes and a few polymorphonuclear leukocytes. Small areas of focal pneumonia and bronchiolitis were observed in both lungs. The sinusoids of the spleen were markedly congested and there were small areas of extravasated blood. Small focal areas of necrosis distributed throughout the liver parenchyma (Fig. 1) were infiltrated with polymorphonuclear leukocytes, red blood cells and large mononuclear cells.

Permission for examination of the central nervous system was not granted.

Cultures procured from the heart's blood and all the viscera yielded a hemolytic Gram-positive bacillus. The ileum and colon also contained this organism. In addition, *Pneumococcus* Group IV and *Hemophilus influenzae* were isolated from the throat.

The unusual nature of the clinical, anatomical and bacteriological findings made it necessary to attempt to determine the source of the infection. The infant's parents proved to be healthy; throat cultures yielded the usual mouth flora and no organisms resembling the one infecting the infant could be isolated. Vaginal examination of the mother 8 days after the birth of the child showed no unusual bacteria. Three of the four children were carefully examined and found to be well. Their throat cultures were entirely negative for pathogenic organisms. No evidence of agglutinins could be found for the organism obtained from the infant with the serum of the mother and father. A pet rabbit found in the house was emaciated and had snuffles, but a more detailed examination of it was refused.

CASE 2. A-3063. The patient was a white, female infant born 3 weeks before the expected date at 8.50 A.M., Feb. 17, 1934, on the outside obstetrical service of the New Haven Hospital. The mother was a 27 year old multipara. This was her fifth pregnancy. There are three children living and well. A spontaneous abortion occurred between the birth of the first two children. Normal spontaneous birth of this child was completed 27 hours after the onset of labor. Repeated stimulation was necessary to elicit regular and continued breathing, after which the child's color became good. However, during the course of the day episodes of labored breathing and cyanosis recurred and the child appeared drowsy. Later, on the 2nd day, after a somewhat prolonged attack similar to those occurring on the 1st day, it was brought to the New Haven Hospital.

On admission the child was drowsy and difficult to rouse. Respirations were irregular and slow. Cyanosis was present. The percussion note was impaired on both sides of the chest anteriorly with dullness in the left axilla. Breath

sounds were poorly transmitted and occasional râles were heard. The heart sounds were of poor quality; the rhythm was regular.

The child continued to have episodes of irregular respirations with complete cessation for short periods and the heart ceased beating in one of these attacks.

### *Pathological Findings*

Postmortem examination was performed one-half hour after death. The body of the infant weighed 2050 gm., and measured 45.5 cm. There was no evidence of skull injury. Both lungs were atelectatic. The spleen was swollen and congested.

Histologically the lungs showed areas of focal pneumonia and bronchiolitis. The liver parenchyma, also, contained foci of necrosis similar to those described in Case 1. However, a few of these foci showed evidence of repair, as manifested by an increase in the fibroblasts within these areas. Both adrenals (Fig. 2) showed histologically evidence of focal necrosis and exudation.

The gross findings in the brain revealed an extensive fresh hemorrhage in the ventricles which extended to the aqueduct of Sylvius and into the fourth ventricle.

Microscopically the blood vessels of the brain were tremendously engorged. There was no evidence of meningitic or encephalitic foci in the cerebral parenchyma.

Clinical postmortem cultures procured at the time of death yielded a hemolytic Gram-positive bacillus from the heart's blood.

CASE 3. A-3074. The patient, a white female infant, was delivered by the outside obstetrical service of the New Haven Hospital on Feb. 17, 1934. It was the fourth pregnancy of apparently healthy parents. The infant was delivered at full term and was born in a normal spontaneous manner, following a labor of 3 hours. She cried and breathed spontaneously and presented no evidence of birth injury. She did well until the 8th day when she rather suddenly refused food. A diarrhea developed in the following 24 hours.

On admission to the hospital there was no evidence of cyanosis, but the patient appeared expressionless and drowsy. The anterior fontanelle was not sunken. Both ear drums were red and bulging but without discharge. The heart and lungs showed nothing unusual. The reflexes showed marked irregularity and at times were absent. Kernig's sign was negative.

The white blood cells increased to 15,450 on the 2nd day after admission, of which 93 per cent were polymorphonuclear leukocytes. There was no evidence of anemia. Repeated daily blood cultures always showed the presence of a hemolytic Gram-positive rod. Spinal fluid, both by direct smear and cultural methods, was positive for the same organism found in the blood stream. Cultures made from the nose, throat, vagina, umbilicus and stools failed to demonstrate the presence of this organism at these sites.

On the 4th day after admission active movements of the extremities devel-

oped which were followed by tonic convulsions. Increasing periods of apnea and cyanosis appeared, the temperature became subnormal, and the child expired on the 5th day of its residence in the hospital and the 14th day of its life.

### *Pathological Findings*

The postmortem examination performed 4 hours after death presented the following findings.

The body was that of a well developed infant weighing 3155 gm., and measuring 54 cm. A few petechial hemorrhages were observed beneath the pleural surfaces of both lungs. Otherwise there was no gross evidence of other change within the lung tissue. The spleen was large, swollen and markedly congested.

Histologically, similar focal zones of necrosis, such as were observed in the previous autopsies, were widely distributed throughout the liver parenchyma. The sinusoids of the spleen were engorged with blood and large hemorrhages were present. The umbilical cord showed no histological evidence of infection.

On gross examination of the brain a thick green exudate was observed in the subarachnoid space covering the medulla, pons and parietal lobes. Both lateral ventricles were filled with a similar type of exudate which extended into the aqueduct of Sylvius (Fig. 3) and completely occluded the lumen. A suppurative ependymitis was observed in both lateral ventricles.

Histologically there was an extensive suppurative meningitis, ependymitis and choroiditis. The process extended into the cerebral parenchyma from the arachnoidal and ependymal linings by way of the vascular sheaths. These vessels were surrounded by an exudate comprised of polymorphonuclear leukocytes, lymphocytes and plasma cells. Hemorrhage was present in all sections. A gliosis (Fig. 4) involving all the glial elements occurred in association with the necrotizing process.

At postmortem a hemolytic Gram-positive bacillus was cultured and demonstrated by stained smears in the heart's blood and viscera, including the brain.

The epidemiological studies of the family showed the mother to have had a cold several weeks before birth of the child. The father and five children were healthy and free of respiratory infection. However, cultures prepared from the posterior pharynx of all the family yielded a few colonies of *Streptococcus hemolyticus* and *Hemophilus influenzae*, besides the usual mouth flora. Likewise,

serological examinations of the family revealed no agglutinins for the organism isolated from the infant. No significant findings were obtained by examination of water and milk used in the household or of the saliva and feces of the pet dog.

CASE 4. A-3167. This patient was a 53 year old, white male who was brought to the New Haven Hospital on June 27, 1934. The immediate illness began in March, 1934, when he developed a bilateral otitis media. Two weeks before admission the left ear became very painful and the patient had a severe headache which persisted until he came to the hospital.

On admission he was uncoöperative and at times irrational. A purulent discharge from the left ear was manifest. There was marked tenderness over the left mastoid. No stiffness of the neck could be elicited. Reflexes were absent and there was a loss of sense of position of the feet. An X-ray examination of the mastoid showed bilateral mastoiditis with evidence of marked destruction of the petrous bone on the left. On the 6th postoperative day, following a mastoidectomy (left), the patient developed signs of meningitis. A second operation revealed an abscess of the petrous apex from which *Pneumococcus* Type III was obtained. The laboratory findings were compatible with an acute infection. The blood became positive for *Pneumococcus* Type III and death occurred on the 9th day after the operation.

### *Pathological Findings*

Postmortem examination performed 2 hours after death presented the following findings.

The body was that of an emaciated white male, weighing 52 kilos and measuring 178 cm. A serosanguineous exudate drained from the operative incision of the left ear. The superficial lymph nodes were enlarged. Both lungs were voluminous because of congestion and edema. Histologically both lungs showed evidence of focal pneumonia and bronchiolitis.

The cortical surfaces of the brain were covered by a thick green exudate within the subarachnoid space. Microscopically this exudate was comprised of polymorphonuclear leukocytes, but showed no extension into the cerebral parenchyma.

The heart's blood and viscera, including brain, contained a *Pneumococcus* Type III. In addition, a hemolytic Gram-positive bacillus was procured from the brain, liver and both kidneys, but not from the heart's blood or spleen. This bacillus was demonstrated in the meningeal exudate by stained smears.

### DISCUSSION

The clinical and anatomical findings in the 4 cases described above have several factors in common. A new pathogen belonging to the

TABLE I

*Summary of Clinical and Postmortem Findings*

No. of Case	Age	Presenting symptoms and signs	Physical examination	Blood culture	Total duration of disease	Autopsy findings				Postmortem bacteriology		
						Lungs	Liver	Spleen	Brain	Heart's blood	Liver	Brain
2810	1 day	Labored breathing, cyanosis, normal delivery	Temp. 101° F., irregular breathing, cyanosis, no jaundice	Hemolytic Gram-positive bacillus	24 hrs.	Focal pneumonia, bronchiolitis, atelectasis	Focal necrosis	Congestion and hemorrhage	Not examined	+	+	—
3063	1½ days	Irregular and weak respiration, cyanosis, drowsy, normal delivery	Temp. 98° F., irregular breathing, cyanosis, no jaundice	Hemolytic Gram-positive bacillus	36 hrs.	Focal pneumonia, bronchiolitis, atelectasis	Focal necrosis	Congestion	Hemorrhage in ventricles	No cultures procured at postmortem		
3074	14 days	Diarrhea, onset on 8th day after birth, normal delivery	Temp. 101.8° F., cyanosis, no jaundice, drowsy, expressionless, apnea	Hemolytic Gram-positive bacillus	6 days	No change	Focal necrosis	Congestion and hemorrhage	Suppurative meningitis	+	+	+
3167	53 yrs.	Bilateral otitis media, operation (mastoidectomy), meningitis	Temp. 98.6° F., tenderness of mastoid, meningitis — 6 days postoperative	Pneumococcus Type III	6-8 days?	Diffuse pneumonia	Focal necrosis	Congestion and hemorrhage	Meningitis	o	+	+

+ = hemolytic Gram-positive bacillus.

o = no growth in bouillon.

— = not cultured.

genus *Listerella* has been readily obtained from the heart's blood or viscera in all 4 cases. The organism has been grown by the usual culture methods and has been demonstrated readily by the Gram stain in the various tissues. Cultural, serological and pathogenic properties of these strains are described in another communication.<sup>1</sup>

In all 4 cases the anatomical lesions involved the liver and in the 3 cases in which the central nervous system was examined lesions were revealed in the tissues. Otherwise, there were no clinical or anatomical manifestations that would permit a differentiation of this infection from other infectious processes without careful bacteriological studies made either during the clinical course of the disease or at postmortem.

Confusion in a bacteriological diagnosis may arise from two standpoints. First, since this bacillus is markedly hemolytic on blood agar plates and in blood broth, and since it has a tendency to form short chains in meat infusion broth, particularly when freshly isolated from the tissues, it may be mistakenly called a *Streptococcus hemolyticus*. Second, it has some of the characteristics of the diphtheroids and consequently may be overlooked as a non-pathogenic organism. Since careful morphological, cultural and pathogenic studies are required for identification of this new pathogen, a more detailed comparative study with some related organism will be forthcoming in a later communication.

The literature contains isolated reports in which Gram-positive bacilli or diphtheroids have been described in association with meningitis. The description of the cultural and pathogenic properties of many of the strains is inadequate but in a few instances sufficient data are available to exclude identification with the organism under consideration. In an epidemic of meningitis occurring in infants, Atkinson<sup>2</sup> isolated a Gram-positive bacillus which may be similar to the one herewith described, although a more detailed cultural, serological and pathogenic study would be necessary before relationship could be established. Schultz, Terry, Brice and Gebhardt,<sup>3</sup> however, have recently described the isolation of an organism from a non-fatal case of meningo-encephalitis occurring in an adult, which has proved to be identical morphologically, culturally and serologically with the hemolytic Gram-positive bacillus isolated from these 4 cases.

The latter fact is of importance since it is hardly a coincidence that

the same organism should be isolated in 4 separate fatal cases here, and also by observers in California. Moreover, Jones and Little,<sup>4</sup> of Princeton, and Seastone<sup>5</sup> recently isolated and described a similar organism associated with a suppurative meningitis in cattle. Through the courtesy of these investigators,<sup>3,4,5</sup> it has been possible to study transplants of their strains and they have been found to be identical, both culturally and serologically, with those isolated from the 4 cases included in this report. A possible source of the infection in man through the milk supply is suggested in view of the isolation of the same organism in suppurative meningitis of cattle.

#### SUMMARY

A hemolytic Gram-positive bacillus of the genus *Listerella* has been isolated from each of 4 fatal human infections. Three of the individuals were newborn infants and the fourth an adult.

The clinical symptoms and anatomical changes presented by the fatal cases are briefly described.

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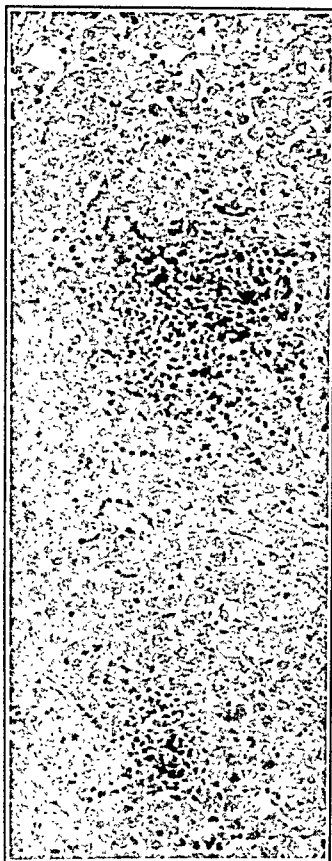
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#### DESCRIPTION OF PLATE

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##### PLATE 39

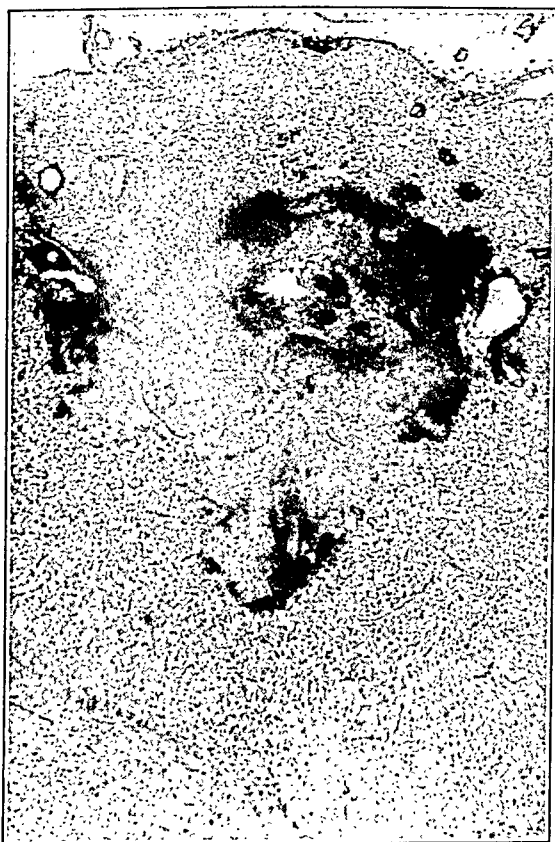
- FIG. 1. Case 1. Focal zone of necrosis in liver.  $\times 70$ .  
FIG. 2. Case 2. Focal zone of necrosis in adrenal.  $\times 70$ .  
FIG. 3. Case 3. Suppurative meningitis with occlusion of fourth ventricle.  $\times 70$ .  
FIG. 4. Case 3. Suppurative meningitis with marked glial proliferation.  $\times 70$ .



1



2



3

Burn



4

New Pathogen of Genus *Listerella*





# MALIGNANT TERATOMA OF THE MEDIASTINUM \*

## REPORT OF A CASE AND REVIEW OF 24 CASES FROM THE LITERATURE

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### I. REPORT OF CASE

This case is of interest because of the rarity of the primary lesion, the somewhat unusual nature of the metastases, and the pertinent associated findings in the testis.

*Clinical History:* I. P. (Hospital No. 720023), a 22 year old, white American farmer, native of Athol, Mass., entered the Boston City Hospital June 6, 1933, complaining of intermittent dry cough for 10 months and stabbing pain in the region of the lower right ribs for 4 weeks. The pain radiated to the upper chest and shoulder and had become so severe as to make it hard to take a breath.

The father died at 62 of "cancer," the mother at 54 of diabetes, and one brother died in infancy. Two brothers and three sisters are alive and well. The patient had had measles, chickenpox, whooping-cough and mumps in childhood, with tonsillitis 6 or 7 years previously. Eight months ago he broke his right wrist, which healed satisfactorily. Six weeks ago he underwent an operation for appendicitis, at which time an appendix showing relatively little pathology was removed. Since then his appetite had been poor, he had lost 25 pounds in weight (165 to 140), and had been running a low fever. Otherwise he had been strong and healthy and "on the go" all the time, except that he had always been "short-winded."

Physical examination revealed a well developed, well nourished young man with bulging of the right chest anteriorly between the third and fifth ribs, right inspiratory lag, flatness anteriorly and bronchial breathing posteriorly. There was a slight inspiratory wheeze. The heart was pushed to the left, and an apical systolic murmur was present. Electrocardiogram showed low T<sub>1</sub> and right ventricular predominance. The urine, blood and Kahn tests were negative. A white blood cell count reached 15,000 on one occasion, temperature not over 101° F. A chest plate revealed a large, rounded tumor mass emerging from the mediastinal shadow into the right chest cavity (middle two-thirds) and a small one emerging on the left.

Two courses of X-ray treatment were ineffective and the patient was readmitted August 13 with a complaint of continued right-sided chest pain and brassy cough. A gradual downhill course was accompanied by low-grade fever (up to 100° F.) and enlargement and tenderness of the liver, edema of the neck, face and arms, dyspnea, orthopnea and cyanosis. The heart and trachea were

\* Received for publication October 15, 1935.

displaced to the left. Pressure on the superior vena cava was evident. A suicidal attempt with razor blades was unsuccessful.

Three chest taps yielded a total of 2400 cc. of amber blood-tinged fluid with a specific gravity of 1.018, total protein 4 mg. per cent, and 11,000 red blood cells. The urine was negative. The blood showed 4,200,000 red blood cells. The hemoglobin was 76. A slight hemoptysis occurred on one occasion.

Abdominal nodules were thought to have been felt but the patient was markedly constipated.

Venous engorgement increased in the neck and face, the scalp as well as the lower legs becoming edematous. Breathing became difficult and stertorous, his strength failed and coma was followed by death Sept. 3, 1933.

The clinical diagnoses were: primary mediastinal tumor, (?) lymphosarcoma, and (?) sarcoma with metastases to liver and abdominal nodes.

Unfortunately the possibility of teratoma was not considered and no hormone studies were done.

### *Postmortem Examination*

Autopsy (No. 33-519) was restricted to examination through an abdominal incision.

*Body:* The body was that of a well developed, well nourished male, 175 cm. in length, with normal masculine habitus. Arms and shoulders were moderately edematous, the face slightly cyanotic. The breasts showed no external abnormality or enlargement suggestive of any degree of gynecomastia. A healed right rectus ap-pendectomy scar was present.

*Peritoneal Cavity:* No fluid, enlarged lymph nodes or tumor masses were seen. The liver was greatly enlarged, the edge 12 cm. below the right costal margin with the diaphragm at normal level. The appendix was absent.

*Pleural Cavities:* 700 cc. of opalescent yellowish fluid were present in the right cavity. A large, rounded, solid, gray tumor mass of predominantly fibrous consistence occupied most of the upper anterior two-thirds of the mediastinum and right pleural cavity, projecting slightly into the left. It measured 18 by 14 cm., was 12 cm. in its greatest anteroposterior thickness, weighed 1590 gm. and was discrete, encapsulated, and firmly fixed to the sternum and anterior wall over an area about 8 by 7 cm., from which it could be freed only with the knife. This mass adhered widely to the upper and right side of the pericardium and hilic region of the right lung, pressed strongly downward on the great vessels and trachea above, overlay the aortic arch, and partly surrounded the right innominate vein. Separation from all but the first and last of these structures was

effected by blunt dissection. Precise observation of the relations was impossible through the abdominal incision.

Several large nodules or lobulations projected from the mass, and at one such point on each side the tissue was hemorrhagic and the capsule frayed. A single, white, fibrous implantation nodule occurred on the lower right parietal pleura laterally (1.5 cm. in diameter) and two more (0.4 and 0.9 cm.) on the lower lobe of the left lung; a fourth and larger nodule (3.5 cm.) on the anterior aspect of the right middle lobe extended well into the lung.

Multiple sections of the tumor mass showed it to be largely solid throughout with no evidence of segmentation or differentiation of organoid complexes. The cut surface presented many fine lobulations of heterogeneous tissues separated by narrow fibrous trabeculae — many yellow fatty spots (0.5 to 1 cm.), scattered hemorrhagic points, an occasional small cyst with clear gray fluid, and rarely a group of projecting nodules of cartilage 1 mm. in diameter. The stroma and bulk of the tissue was fibrous, pinkish gray, bulging and resilient.

*Heart:* Slightly subnormal in size (weight 260 gm.). The pulmonary and aortic valve orifices were proportionately small (5.8 and 5.4 cm. in circumference respectively). Traces of atheroma were present in the coronary arteries and above each aortic sinus. No ventricular hypertrophy was present.

*Lungs:* Weight of right 340 gm., left 480 gm. The right lung was small, the lower lobe almost totally atelectatic (believed to be due to hydrothorax); the upper lobe was pink and crepitant. Three tumor nodules were present, as noted. An encapsulated, fibrous tumor mass 4 by 1.3 cm. was present lying along the bronchus of the right middle lobe, apparently a metastasis arising in the peribronchial lymphatics. The left lung was crepitant throughout, with some congestion at the base. The trachea and bronchi were moderately reddened.

*Spleen:* Weight 210 gm. Slightly firm and cut surface dark red.

*Gastro-Intestinal Tract:* Negative.

*Pancreas:* Negative.

*Liver:* Weight 3900 gm. A single, large, round circumscribed metastasis, 15 cm. in diameter, was present in the upper outer part of the right lobe, where it approached the capsule but produced no deformity of the organ. The tissue was fibrous, pinkish gray,

bulged from the cut surface and was concentrically arranged. A few, slightly hemorrhagic areas, and a single, sharply defined yellow band of necrosis were seen centrally. Rare bits of cartilage and bone or calcified material were felt. The liver tissue was a uniform pale brown color.

*Kidneys:* The combined weight was 365 gm. Marked congestion was present.

*Adrenals:* Small.

*Bladder and Ureters:* Negative.

*Prostate:* Not enlarged. No histological examination was made.

*Testes:* Of equal size, firm and symmetrical, but distinctly small (2.3 by 1.4 cm.). The cut surface was a uniform pale brown. The tubules failed to string out properly when picked up with the forceps. A single median section was made through each testis, and a few additional cuts. From the uniform pliable consistence and evenness of the thin halves when laid flat it was felt most probable that no tumor of other than decidedly minute proportions could be present.

*Penis:* Of normal size.

*Aorta:* Narrow and thin-walled throughout. A small patch of atheroma was present in the lumbar portion.

*Vertebral Marrow:* A poorly demarcated grayish area 2 cm. in diameter was present in an upper lumbar vertebra. Elsewhere the marrow was a uniform red color.

Because of the autopsy restrictions no examination was made of the thyroid, brain or pituitary. X-rays of the humeri, bones of both shoulders, femora and pelvis showed no abnormality.

### *Microscopic Examination*

*Heart:* Negative. The muscle fibers are narrow, the nuclei not swollen.

*Mediastinal Tumor:* Teratoma of complex type with derivatives of all three embryonic layers. It consists largely of fairly well differentiated fibrous stroma, mesenchyma-like in places, through which are scattered a variety of other tissue elements. Islands of fetal cartilage and solid acini of stratified epithelium are prominent. The stroma shows none of the signs of rapid growth, and in a few places contains fairly dense hyaline collagen which has presumably been present for a considerable time.

Other elements found include acini of columnar epithelium of intestinal type (with goblet cells), thickly stratified epithelium of neural tube type with an inner ciliated layer, ganglion cells and nerve fibers, bone and osteoid tissue, adult and embryonic fat, cystic spaces lined with simple cuboidal mesothelium, and endothelium-lined spaces with associated smooth muscle or hyaline material. In one small area narrow epithelial cords with occasional mitotic figures are growing in young fibrous stroma, suggesting scirrhus carcinoma. Other parts show edema, necrosis or hemorrhage, usually of slight degree.

No areas of either chorionepithelioma or embryonal cell carcinoma are found.

*Right Peribronchial Metastasis:* The appearance is similar to the main tumor: fibrous stroma, cartilage, bone, fat, mesothelium-lined spaces, and five different types of epithelium, including intestinal, are present.

*Lung:* Areas of bronchopneumonia are seen. Alveoli in places are lined with a thick layer of eosinophilic material, perhaps dried serum.

Tumor implant of complex constituents as above, including cartilage, intestinal epithelium, and so on, is present.

*Spleen:* Slight chronic passive congestion is present.

*Liver:* Metastases, again of the same complex type, composed chiefly of slowly growing fibrous stroma and containing most of the elements found in the mediastinal mass are seen. The liver parenchyma is normal.

*Pancreas, Kidney and Adrenal:* Essentially negative.

*Testis:* Marked atrophy of tubules and hyperplasia of interstitial cells are present in both testes. The atrophy is of the post-pubertal type, most of the tubules showing complete disappearance of both spermatogonia and supporting cells; the tubules persist in only very few places and no spermatozoa are present. From one to four tubules with persistent supporting cells occur in about two-thirds of the lower power fields (10 × objective). The interstitial cells extend diffusely among the tubules (Fig. 3), and frequently form large solid nodules up to 1.5 mm. in diameter (Fig. 4).

*Vertebral Marrow:* The gray area noted consists of a single complex teratomatous metastasis, of the same type as the others, extending centrifugally among the bone spicules with a poorly defined

advancing margin and no encapsulation. Intestinal epithelium is present, also fetal cartilage, cystic spaces, connective tissue, and so on. The myeloid tissue is normal.

### *Comment*

The complex constitution of the various metastases in this case indicates dissemination at one time or another from a primary focus of essentially totipotential cells.

In view of the considerable uniformity in degree of differentiation and apparent rate of growth in the different metastases, the relatively small size of the pleural implants might be taken to indicate that they are of comparatively recent origin.

If such be the case, totipotential cells must have persisted as part of the main tumor. We do not, however, find any cells of totally undifferentiated appearance in the existing primary mass. The less differentiated spindle cells of the stroma would seem to be the only ones that could possibly be implicated.

Was the bilateral testicular atrophy due to mumps? The chances are 23 to 1 against a complicating orchitis in this disease when it occurs during childhood.<sup>1</sup> Bilateral involvement would be still more unlikely. Secretion by the tumor of a hormone or hormones directly or indirectly antagonistic to the spermatogenetic tissue is a more probable cause.

### *Résumé*

A 22 year old white male with a history of 10 months duration of chest symptoms (and of always having been "short-winded") develops mediastinal obstruction and eventually dies, apparently from mechanical strangulation by a large tumor. Autopsy reveals a 1590 gm. solid teratoma of the mediastinum, with pleural implants and metastases of a uniformly complex type in lung, liver, vertebral marrow and a peribronchial node. There is an associated marked atrophy of the tubules of both testes with great interstitial cell hyperplasia. No chorioneplithelioma occurs either in the primary tumor or in any of its metastases. The heart, aorta, adrenals and testes are of small size. A right hydrothorax is accompanied by atelectasis of the right lower lobe.

## II. REVIEW OF LITERATURE

The subject of intrathoracic dermoid cysts and teratomas (191 cases) has been reviewed with exceptional thoroughness by Hedblom.<sup>2</sup> The increasing number of reports in the literature tend to give a false idea of the frequency of the condition. Hare<sup>3</sup> found only 11 cases out of 288 mediastinal tumors (including secondary metastatic growths). Of 155 thoracic tumors in the Chest Tumor Registry up to February, 1935,<sup>4</sup> there were 7 dermoid cysts, of which 1 was a true teratoma.

Collections of the reported cases, with brief details of each, and summaries covering the age and sex incidence, symptoms, location, course and prognosis of these tumors, have been made by Morris,<sup>5</sup> Hertzler,<sup>6</sup> and Lambert and Knox.<sup>7</sup> A classification according to structure from the study of 100 cases is given by Williams.<sup>8</sup>

### *Theories of Origin*

Most extragenital teratomas, according to Ewing, probably arise from aberrant sex cells, which may occur anywhere along the entire length of the embryonal entoderm.<sup>9</sup> On the other hand, origin from an isolated blastomere (one of the cells or groups of cells in the morula resulting from the earlier divisions of the fertilized egg) would account for the general or special tissue constitution of a growth by assuming earlier or later isolation. Aberrant sex cells, however, have actually been found in the embryo, whereas isolated blastomeres of this sort have not been identified.

Origin by inclusion of a fertilized polar body or of a second fertilized ovum (bigeminal origin) has been proposed, but according to Budde<sup>10</sup> a true fetal inclusion should develop metameric segmentation, which has not thus far been observed in any intrathoracic case. A supposed fetal inclusion is described by Harrington.<sup>11</sup>

Fortuitous budding of superfluous tissue in the embryo probably accounts for certain tumors or rests composed of local tissue complexes only.

The testis and ovary are the commonest sites of teratomas. Functional accessory testicular tissue has been found at the root of the mesentery by Staemmler.<sup>12</sup> Symeonidis<sup>13</sup> has described a retroperitoneal chorionepithelioma apparently arising from a super-



numerary abdominal testis, and Staemmler considers the possibility of mediastinal tumors arising from an intrathoracic testis. Whether or not accessory testicular tissue may develop as a compensatory hyperplasia resulting from testicular atrophy remains to be demonstrated. At all events no testicular tissue has as yet to my knowledge been found in the thorax, either by itself or as part of a teratoma.\*

Origin of mediastinal teratoma by metastasis from a minute primary testicular tumor is discussed below.

### *Relationship of the Different Forms*

Hedblom distinguishes three types of teratoma: (1) the epidermoid cyst, containing only ectodermal derivatives; (2) the dermoid type, from ectoderm plus mesoderm; and (3) the true teratoma, from all three embryonic layers. Of 120 cases with microscopic examination, 42 were of the first type, 40 of the second, 38 of the third. Estimated by the gross appearance of additional specimens, more than half of the 184 classified cases were purely epidermoid.

The principle of overgrowth of one type of tissue with suppression of others probably accounts for many unidermal tumors which presumably started from totipotential sex cells. Since the early embryo is largely composed of ectoderm, overgrowth of this layer is believed a natural consequence (Ewing).

Probably occasional purely mesodermal and entodermal tumors of teratomatous derivation in the mediastinum have passed as of unexplained source. Among these may be placed some of the fibromas, cystic lymphangiomas, complex sarcomas, and undifferentiated blastomas of the literature. (In the testis all chondromas are teratomatous according to Ewing.)

Intergradation among teratomas occurs not only in the number of tissues present (simple to complex), but also to a considerable extent in the gross form (simple cysts, multilocular cysts with solid areas, and solid masses with small cysts). It likewise occurs with respect to the degree of differentiation and rapidity of growth. Differences in the last two characteristics occur even in the same tumor, in which areas of embryonal cell carcinoma or of chorion-epithelioma may be found along with a complex adult type of growth.

\* Ovary-like tissue was found by Gordon.<sup>14</sup>

Chorionepithelioma, according to Ewing, is merely the embryonal equivalent of adult ectoderm. True extragenital chorionepithelioma probably always arises in teratomatous tissue.<sup>15</sup>

A genetic continuity may thus be conceived between the various types of growth. This concept is further supported by physiological evidence in the work of Ferguson,<sup>16</sup> who found prolan excretion in all actively growing types of testicular teratoma, varying according to the degree of differentiation.

### *Malignancy*

Askanazy<sup>17</sup> divided teratomas into two classes: (1) an adult or coetaneous type, cystic, composed of well differentiated tissues of an age apparently equal to that of the host; and (2) an embryonal type, solid, of young growing tissues with great inherent power of proliferation. The first type, illustrated by the common ovarian dermoid, becomes malignant by late secondary degeneration, usually as squamous cell carcinoma, which is thus a "tumor within a tumor." The second type may behave at first as a benign tumor but grows on until pressure effects, invasion, or metastases supervene. The metastases may be complex, with all the tissue components of the primary tumor, or composed of one or two elements only. The case presented above fits fairly well into the second category, but it must be admitted that this classification is useful only as a generality. Willis<sup>18</sup> has pointed out that intermediate forms exist and no sharp distinction can be drawn.

### *Intrathoracic Cases*

Hedblom found that in 191 dermoid cysts and teratomas 17 (8.9 per cent) were malignant. Subsequently reports of 25 more dermoids have been published, including this one, making 216 in all. I have found in the recent as well as the older literature 24 cases of malignant intrathoracic tumors originating in (or at least very closely associated with) teratoid tumors, to which the case here reported is added, making 25 in all.

They are arranged here in groups roughly according to gross characteristics of the primary tumor (solid or cystic) and type of metastasis, if any. No review of the histological material has been

made, nor has any grouping into embryonic or adult types been attempted on the basis of the histology described, inasmuch as there is no uniformity in the method or thoroughness with which the tumors were examined.

*Group I. Cases with Solid or Predominantly Solid Tumors*

*(A) With Metastases*

*(1) of Multiple Components*

1. Virchow.<sup>19</sup> Male. Age 22 years. Solid tumor 20 by 21 by 13 cm., the smaller half cystic. Complexity of tissue elements. Areas of sarcomatous and carcinomatous appearance. Metastases to liver, spleen, kidneys, and third left rib. Rib metastasis of many elements, as in primary tumor; had grown to pigeon's egg size in 5 weeks.

2. Becker and Carey.<sup>20</sup> Male. Age not given. Solid tumor 30 by 20 by 10 cm. Very cellular — cartilage, mesenchyma, glands, areas of necrosis and hemorrhage. Liver nodules (1 cm. in diameter) composed of loose reticulum with cysts and glands of cuboidal, columnar and ciliated epithelium.

3. Houghton. Male. Age 22 years. Solid tumor 18 by 14 by 12 cm. No definite histological malignancy. Metastases of complex nature in pleura, lungs, bronchial node, liver and vertebral marrow. (Reported above.)

*(2) of One Component Only*

4. Warthin.<sup>21</sup> Male. Age 26 years. Solid tumor size of child's head. Complex, with nervous tissue, rudimentary teeth, cartilage, and so on. Cellular areas, part sarcomatous and part carcinomatous; some "syncytial formation." Pericardium and epicardium studded with carcinomatous nodules.

5. Bull.<sup>22</sup> Male. Age 17 years. Solid tumor 15 by 15 cm. with several egg sized cysts. Complex. Areas of irregular epithelial proliferation. Metastases to lungs, liver, supraclavicular, infraclavicular and left axillary nodes as adenocarcinoma.

6. Pol.<sup>23</sup> Male. Age 28 years. Solid tumor 12 by 15 by 7 cm., with smaller area of cysts up to pea size; smooth muscle, cartilage and ciliated epithelium, without epidermal elements. Solid epithelial cell nests; stroma sarcoma-like in places. Metastases to lower cervical nodes: "epithelial blastoma, probably adenocarcinoma."

7. Schütt.<sup>24</sup> Male. Age 19 years. Solid tumor 17 by 12 by 11 cm., partly soft and hemorrhagic. Complex. Adenocarcinoma and alveolar sarcoma, both invading vessels. Implants on the right lung, nodules on pericardium (histology not described).

8. Rolleston.<sup>25</sup> Male. Age 20 years. Soft, solid, hemorrhagic, partly cystic tumor 7 by 6 inches (about 18 by 15 cm.). Cartilage and bronchial epithelium, sarcomatous areas of small round cells and oval or spindle-shaped cells (some "possibly epithelial"). Diagnosis: "hemorrhagic adenochondrosarcoma." Invasion of pericardium and superior vena cava. A few hemorrhagic nodules in the lungs. Testes small; microscopically "interstitial increase and thickening of the basement membrane of the seminiferous tubules."

9. Rose.<sup>26</sup> Male. Age 55 years. Solid tumor 23 by 14 by 12 cm., with central degeneration and cyst-like cavities. Cartilage, small cysts with ciliated

epithelium. Cellular sarcomatous areas; cells irregular, moderate sized with pale nuclei and abundant cytoplasm. Multinucleate cells resembling Dorothy Reed type. Metastases to lungs, pleura, pericardium, regional nodes, thyroid and left adrenal. "Probably thymic" in origin.

(B) *Without Metastases*

10. Lindstedt.<sup>27</sup> Male. Age 32 years. Solid tumor 22 by 18 by 9 cm. Complex. Brownish mushy areas. Round cell, spindle cell and alveolar sarcoma, adeno- and medullary carcinoma. Syncytial cells. Areas of necrosis. Superior vena cava invaded, containing a tumor mass 4 cm. long composed of loose papillomatous connective tissue and cysts (up to pea size) lined with high columnar epithelium.

11. Sieber.<sup>28</sup> Male. Age 16 years. Partly solid tumor 19 by 22 by 11.5 cm., with cysts up to walnut size. Complex. No hair or teeth. Carcinomatous and sarcomatous areas. Poorly demarcated from left upper lobe with questionable invasion.

12. Stein.<sup>29</sup> Male. Age 25 years. Solid tumor 14 by 7 by 6.5 cm. Very complex. Spindle cell sarcoma with marked polymorphism and giant cells. Rich in blood vessels. Colloid carcinoma with signet ring cells. Red necrotic areas.

*Group II. Cases with Cysts or Predominantly Cystic Tumors*

(A) *With Metastases*

(1) *of Multiple Components*

13. Jores.<sup>30</sup> Male. Age 39 years. Cystic tumor 31 by 20 by 16 cm. Hair and sebaceous material present. Large cyst 19 by 6 cm. with squamous epithelial lining; second fist sized cyst with teeth, solid areas with cartilage, sweat glands, and so on. Local mass and nodules in lungs and pleura of spindle cell sarcoma in which were cystic spaces lined with high columnar epithelium (only two components).

(2) *of One Component Only*

14. Ceelen.<sup>31</sup> Male. Age 33 years. Dermoid cyst of goose egg size with solid parts. Hair, sebaceous material, skin, cartilage, smooth muscle, spaces with epithelium and colloid material suggesting thyroid. Adenocarcinoma in wall, with metastases of similar histology in pleura, lungs, cervical and bronchial nodes and liver. Invasion of sternum, where tumor was growing as squamous cell carcinoma.

15. Jacobs.<sup>32</sup> Male. Age 27 years. Cyst 14 by 9 by 8 cm. with wall 0.2 to 3 cm. thick. Hair and sebaceous material. Complex. Metastases of adenocarcinoma in lungs, thyroid, cervical and tracheobronchial nodes and meninges. Testes grossly normal; histology not described.

16. Singer.<sup>33</sup> Female. Age 38 years. Partly cystic tumor 14 cm. in diameter. Hair in one cavity. Complex, with bone spicules and papillary cystic formations. Metastases in lungs (histology not described).

17. Pinders.<sup>34</sup> Sex and age not given. Cyst of goose egg size containing fatty material, 17 cm. in diameter. Solid part composed of lymphosarcomatous tissue. Invasion and metastases in lungs. Possible thymic origin. Cyst wall fibrous and calcified.

(B) *Without Metastases*

18. Harrington.<sup>35</sup> Male. Age 49 years. Multilocular cystic tumor 20 by 18 by 15 cm. Wall fibrous, with squamous cell carcinoma *in situ*. Complete removal at operation.

19. Terplan.<sup>36</sup> Male. Age 41 years. Cystic tumor, size greater than fist. Hair, bone, derivatives of all three embryonic layers. Area of carcinoma the size of a small pea. Two pea and bean sized nodules (dermoids?) in the left interlobar septum, fibrous and calcified.

20. Eiselsberg.<sup>37</sup> Child, age 3 years, sex not stated. Dermoid cyst containing bone and cartilage. "Sarcomatous degeneration."

*Group III. With Chorionepithelioma \**

21. Ritchie.<sup>38</sup> Male. Age 24 years. Solid, large hemorrhagic mass 15 by 12 by 6 cm. continuous with a dermoid cyst 7 cm. in diameter containing hair and sebaceous material. Subsidiary cysts, myxomatous connective tissue and fat tissue. Solid part pure chorionepithelioma; metastases as such in lung, liver and spleen. "Other organs of body normal."

22. Lambert and Knox.<sup>7</sup> Male. Age 34 years. Solid, cocoanut sized mass; main part honey-combed with small cysts. Very complex teratoid structure. Chorionepitheliomatous areas, chiefly in anterior portion, readily located by associated hemorrhage. Metastases of pure chorionepithelioma in lungs, bronchial nodes, parietal pleura and liver.

23. Kantrowitz.<sup>39</sup> Male. Age 22 years. Solid tumor 8 by 5 by 4 cm. Composed entirely of chorionepithelioma except for a complex multicystic teratomatous area in the lower pole. Invasion of superior vena cava and left innominate vein. Lungs full of chorionepitheliomatous metastases. Marked interstitial cell hyperplasia of testes, with atrophy of numerous tubules. Microscopic glandular hyperplasia of prostate. No gynecomastia.

24. Hammarskjöld.<sup>40</sup> Male. Age 22 years. Cystic, complex teratoma, fist sized, with chorionepitheliomatous growth metastasizing to lungs.

25. Arendt.<sup>41</sup> Male. Age 20 years. Mass larger than fist, of solid chorionepithelioma with a central, round, laminated structure of cornified epithelium. Invasion of pericardium. Metastases to lungs and liver. Lymph nodes not involved. Interstitial cell hyperplasia of testis, almost tumor-like; complete atrophy of tubules. Small adrenals. Female habitus. Marked gynecomastia had developed within only 15 days.

*Unproved or Unaccepted Cases (Not Included in Series)*

1. Smith and Stone<sup>42</sup> found in a boy of 20 months solid posterior mediastinal masses of "papillary cysto-carcinoma" with invasion of the diaphragm and retroperitoneal extension. The stroma resembled embryonal mesenchyma. Conceivably teratogenous. Origin not determined.

2. Harrington.<sup>43</sup> Female. Age 59 years. Large tumor diagnosed cystic teratoma by X-ray. Bulging of chest wall for 30 years, increase in size within

\* Syncytial formation, multinucleate cells, two types of cells, or gross hemorrhagic areas are also described in the cases of Warthin, Schütt, Rolleston, Lindstedt, Stein and Rose.

1 year. Biopsy of supraclavicular node showed squamous cell carcinoma. No confirmation of X-ray diagnosis either by operation or autopsy. Source of carcinoma not established.

3. Fawcett.<sup>44</sup> Female. Age 62 years. Solid tumor (type and histology not given) with white growth infiltrating the lung. Lower part softened, forming an orange sized cyst. Mediastinal glands enlarged with growth.

4. Griffin.<sup>45</sup> Male. Age 15 years. Partly cystic tumor. Solid masses 16 by 8 by 7 and 8 by 8 by 6 removed at operation. Innumerable small cysts, cellular connective tissue of fetal type. Histologically appeared "probably not very malignant." Clinical recurrence (marked bulging of chest) in 2½ months. No autopsy. Recurrent tumor presumed to be teratoma.

5. Loewenmeyer.<sup>46</sup> Male of unspecified age. Tumor of the size of a child's head occupying most of the left chest, adherent to lung, pericardium, diaphragm; contained cysts, hair, cartilage, and so on, and "sarcomatoid" and "carcinomatoid" tissue (Williams<sup>8</sup>). Thrombosis of right innominate vein because of compression. No mention of extracapsular extension or frankly malignant histology.

6. Black and Black.<sup>47</sup> Male. Age 46 years. Tumor largely solid; cystic portion containing fluid and cheesy material encountered at operation. Biopsy showed teratoma with "a few carcinomatous cells." No autopsy.

7. Helbing.<sup>48</sup> Male. Age 23 years. Solid tumor 21 by 10 by 10 cm. in position of left lung. Complex, with small epithelial cysts, cartilage, giant cells, lymphoid cells. Extensive growth as "rhabdomyoma."

8. Pohl.<sup>49</sup> Female. Age 1 year. Solid mass (with small cysts) 12 by 5 by 4 cm. in the right upper lobe region; extending around (but not invading) the great vessels. Complex, with well differentiated tissues, hair and sebaceous material. Proliferating epithelial structures but no definite histological evidence of malignancy.

9. Krassnianskaya.<sup>50</sup> Male. Age 72 years. Mass of chorionepithelioma at hilum of left lung. Metastases in many organs including retroperitoneal nodes. No teratoma found. Although the testes were excluded as a source by examination in very thin sections, a retroperitoneal origin appears possible. The hilic mass could perhaps have arisen by extension from metastasis in a node.

10. Frank.<sup>51</sup> Male. Age 21 years. Necrotic mediastinal mass with metastases of chorionepithelioma in lungs and liver. No teratoma found. Probably primary in the mediastinum, but this could not be proved because of failure to examine the testes.

### *Comment*

In Pinders' case it is possible that the lymphosarcoma arose in the thymus quite independently of the dermoid cyst, which would then have become involved only because of its close juxtaposition.

Histological demonstration of the malignant element arising within the teratoma appears to have been lacking in the cases of Jacobs and Singer; it is best described in those of Ceelen and Harrington. Bronchiogenic carcinoma should be carefully ruled out wherever the malignant element is epithelial.

Location of the primary tumor on or in the position of the lung,

rather than chiefly within the mediastinum, occurs in the cases of Harrington and Eiselsberg (also Helbing and Pohl).

A number of tumors of grossly benign appearance (cases of Loewenmeyer, Black and Black, Griffin, Helbing, Pohl) have shown microscopically areas of rapidly growing embryonic tissue more or less simulating malignancy. Perhaps the case of Terplan also belongs in this category. Diagnosis of malignancy in the usual sense should be made with particular caution in these tumors.

The tumor in Jores' case may be cited as an example of an intermediate form between Askanazy's two classes — it is cystic, with well differentiated elements of the adult type (teeth, hair, sebaceous material), yet exhibits metastases of two components as in the embryonic tumors.

The possible danger of incomplete surgical removal is seen in the rapidly recurrent and fatal growth in Griffin's case.

### Age

The average age in 23 of the 25 cases was  $27\frac{1}{2}$  years. (In 2 no age was given.) The youngest was 3 years, the oldest 55 years. In 14 cases it was between 16 and 28 years. Four of the 5 chorionepithelioma cases were between 20 and 24 years.

The distribution is further shown as follows:

0-4	5-9	10-14	15-19	20-24	25-29	30-39	40-49	50-59
1	0	0	3	7	4	5	2	1

In Hedblom's series (both benign and malignant teratomas) the age was between 17 and 30 years in  $52\frac{1}{2}$  per cent of the 175 cases given, comparing with 61 per cent for this group. A much larger proportion (14.8 per cent) of his cases were in the youngest group (0 to 12 years).

In teratomas of the testis (61 cases of Chevassu<sup>52</sup>), 38 per cent were between 20 and 30 years, whereas 29.5 per cent were between 30 and 40 years — a somewhat older incidence.

### Sex

Only 1 of 23 cases was a female. (The sex was not specified in 2.) More than half (53.8 per cent) of Hedblom's were in females.

All of the chorionepitheliomas were in males.

### *Duration*

Because most of these tumors are operated on as soon as discovered, observations over a period of years to determine the rate of growth have rarely been made.

None of the cases in this series was followed by repeated X-ray examination for any significant length of time, and the duration, as indicated by symptoms or signs, is quite variable and unreliable. In 12 cases it averages  $4\frac{1}{4}$  months, varying from 2 weeks to a year. This period represents the final onset of definitely attributable symptoms leading up to terminal hospitalization. Actual duration is undoubtedly a matter of years in many if not in the great majority of the cases. Three had suspicious but not definitely attributable episodes 3, 6, and 19 years previously (Jores, Terplan, Lindstedt). In 3 additional cases (Bull, Singer, Harrington), the duration was 2, 5, and 4 years respectively, bringing the average for all the available non-chorioneplitheliomatous cases to slightly over 12 months. Benign teratomas have been present 10, 13, 24, and 44 years (Morris).

Average duration of symptoms in the 5 cases with chorioneplithelioma was about  $9\frac{1}{2}$  months, varying from 3 to 15.

### *Size of Tumor*

The following statements apply to the non-chorioneplitheliomatous tumors in adult or adolescent individuals.

The smallest, fatal non-metastasizing tumor was that of Stein, 14 by 7 by 6.5 cm. (the size of "two fists").

The largest tumor of the series was that of Jores (31 by 20 by 16 cm.) which included a cyst 19 by 6 cm. and solid malignant portions.

The smallest metastasizing tumor was of goose egg size (8 cm.), that of Ceelen.

Taking the average of the given dimensions of each tumor as the mean diameter, the average mean diameter in 16 cases was 15.6 cm. Of these 16, 12 metastasized and 4 did not. The average mean diameter of the non-metastasizing cases was 15.1 cm. Thus, the metastasizing tumors are found to be actually slightly larger than the non-metastasizing at time of death, confirming clinical evidence



that it is the size of the primary mass that is of outstanding importance, producing lethal obstructive effects.

The chorionepitheliomatous tumors varied from fist to cocoanut size, the mass in the latter case being composed mainly of solid teratoma.

### *Gross Form*

Of 20 tumors without chorionepithelioma, 8 were essentially solid; small cysts, if present, were no greater than pea to hen's egg size. Three were predominantly solid, 3 predominantly cystic, and in 5 there was a single main cyst; to the latter may be added Pinders' case in which the primary cyst had been overgrown by lymphosarcoma tissue until the mass was chiefly solid.

The cases are distributed as follows:

*Solid:* Becker and Carey, Houghton, Warthin, Bull, Pol, Schütt, Lindstedt, Stein.

*Predominantly Solid:* Virchow, Rolleston, Rose.

*Predominantly Cystic:* Jores, Sieber, Singer.

*Cysts:* Ceelen, Jacobs, Harrington, Terplan, Eiselsberg, Pinders.

Fourteen of these 20 tumors had proved metastases; 9 were solid or predominantly so, and 5 chiefly cystic.

The experience of Askanazy and the observations of Christian<sup>53</sup> and Hörnicke,<sup>54</sup> to the effect that the solid type is more likely to become malignant, are thus further supported.

In the cases with chorionepithelioma a solid teratoma was present in 1, cystic tumors in 2. In 2 others only small areas of teratomatous tissue were present — solid tissue in 1, an essentially cystic structure in the other.

### *Nature of Metastases*

Correlation between gross form and histology of metastases is afforded by the following table:

	<i>Solid Group</i>	<i>Cystic Group</i>
Complex.....	3	0
Two components.....	0	1
Adenocarcinoma.....	2	2
Carcinoma, unclassified.....	1	0
Lymphosarcoma.....	0	1
Sarcoma of questionable type.....	1	0
Histology not described.....	2	1
Chorionepithelioma.....	2	3

### *Secondary Degeneration*

In only 2 cases, those of Harrington and Pinders, does secondary malignancy appear to have occurred in a cyst of long standing. In those of Ceelen, Jores, and Singer, while all in a slightly older age group, the tumor has a complex structure suggesting the embryonic type. In such intermediate cases, positive distinction between secondary degeneration and the final escape of one embryonic element would offer considerable difficulty. It becomes evident that cystic form is no criterion against the embryonic kind of malignancy. Eiselsberg's case ("sarcomatous degeneration" of a dermoid cyst in a child of 3) may well be of this sort.

### *The Testis as a Possible Source*

Symeonidis<sup>13</sup> has emphasized the importance of very careful examination of the testes in cases of supposed extragenital chorionepithelioma. Extensive and large metastases may come from a very small primary testicular tumor. In his own case (a male aged 37 years) he found in the right testis a cystic complex teratoma the size of a small cherry which had metastasized to periaortic and peribronchial nodes, the former then giving rise to chorionepithelioma which invaded the vena cava and metastasized to the lungs. Ewing,<sup>55</sup> in a male, 32 years of age, found pea sized nodules of tridermal structure extending up along the spermatic cord from a very small tumor in the rete; there were abdominal nodes 2 to 6 cm. in diameter and a mass 10 cm. in diameter, with several smaller ones, in the mediastinum (posteriorly, however). Chorionepithelioma was present in the rete, with many metastatic nodules in the lungs and a mass 7 cm. in diameter in the liver.

A large mediastinal tumor found 3 years after removal of a testicular teratoma is reported by Hammarskjöld.<sup>40</sup> The appearance by X-ray was consistent with cystic teratoma.

The finding by Prym<sup>56</sup> of spontaneous regression of a small primary testicular chorionepithelioma renders suspicious the presence even of a small scar.

Appreciation of these observations, including the fact that metastases themselves may be cystic, casts a possible doubt on the primary nature, not only of all mediastinal chorionepithelioma but of all mediastinal teratomas as well, in those cases in which the

testes were not examined. Unfortunately in this series there is specific mention of such examination in only 5 cases (Jacobs, Rolleston, Kantrowitz, Arendt, and our own).

In 2 of these, however (Kantrowitz, Arendt), very careful examination was made and no tumor found. Symeonidis accepts both as apparently primary in the mediastinum.

As pointed out by Kantrowitz,<sup>39</sup> not one example of metastasis to the anterior mediastinum is mentioned in Greiling's series of 220 metastasizing testicular tumors, although the retroperitoneal nodes were involved in every case.

No mention was made of any evidence of tumor in the abdominal lymph nodes at autopsy in any of the 25 cases in this series. Abdominal examination was made in at least 15.

Of 99 mediastinal teratomas that have been operated on, all but 16.1 per cent have been cured or improved (Hedblom). Subsequent appearance of a testicular tumor or of other metastases has not to our knowledge been reported in a single instance.

Metastasis from the testis as a source of these tumors must therefore be exceedingly rare.

### *Interstitial Cell Hyperplasia*

Interstitial cell hyperplasia of the testis has been found associated with varying degrees of atrophy of the tubules in many conditions, including senility, carcinoma, cachexia and tuberculosis (Kaufmann). It has also been found in cases of hermaphroditism, ovario-testis, cryptorchidism, ectopic testis (especially when bilateral<sup>57</sup>); in the offspring of crossed pea and guinea fowl; in certain animals (having a well marked sexual cycle) during the period of sexual activity; in rats fed with anterior pituitary lobe substance; and in experimental partial (or unilateral) castration.<sup>58</sup> Its occurrence following vasectomy or ligation, as found by the earlier investigators, has since been shown by quantitative methods to be apparent rather than absolute.

That it does not arise simply as a mechanical spatial compensation for the shrinkage of the atrophic tubules should be apparent at least in this case from the autonomous character of the tumor-like nodules (Fig. 4) which do not adapt themselves to the existing structure of the tissue.

That it can occur in the complete absence of any tubular atrophy is shown in Hedinger's case of testicular chorionepithelioma.<sup>59</sup>

Interstitial cell hyperplasia with testicular teratoma was a constant finding in the contralateral testis in all of Ferguson's 5 cases with high prolan A excretion. In the series above it was present in the cases of Rolleston, Kantrowitz, Arendt, and our own, being quite marked in the last 3.

Hormonal relations of the interstitial cells to the pituitary and prostate have been indicated in the experiments of Lower.<sup>60</sup> Changes in the pituitary, prostate, seminal vesicles, adrenal and male breast coexisted with interstitial cell hyperplasia in some of Ferguson's testicular teratoma cases. In the above series, however, prostatic hypertrophy was mentioned in only 1 case (Kantrowitz). Small adrenals were noted in Arendt's case and in our own; small testes in ours and in Rolleston's.

In Arendt's case there was complete atrophy of the testis tubules.

The occurrence of interstitial cell hyperplasia in intersexes suggests the proposition that hormone-secreting teratomas may bring about to some degree a comparable physiological condition in persons originally of normal male sexuality.

At all events, the case presented above affords circumstantial evidence that teratoma of the mediastinum has an effect on the interstitial cells of the testis similar to that of teratoma of the testis, and that the presence of chorionepithelioma or of embryonal cell carcinoma is not necessary to produce this effect.

#### SUMMARY AND CONCLUSIONS

1. A case of solid teratoma of the mediastinum with distant complex metastases is described.

2. Theories of origin of teratoma are briefly reviewed and the genetic relations of the different types pointed out.

3. Twenty-four other cases of malignant mediastinal or intrathoracic teratoma have been collected from the literature, together with 10 doubtful cases that have not been included in the statistics.

4. The age of greatest incidence is in the early twenties.

5. Chorionepithelioma was present in 5 of the 25 cases, and had metastasized in every instance. The primary teratoma was overgrown and obscure in 2.

6. Of 23 cases in which the sex was given, 22 were males. All of the patients with chorionepithelioma were males.

7. The size of the primary tumor is of outstanding importance as a cause of death. In 16 cases the average diameter was 15.6 cm.

8. Large size of the primary tumor is no criterion against the occurrence of metastases. Although chorionepithelioma arose from smaller tumors, other types of metastasis came from tumors of size slightly larger than average.

9. Cysts may become malignant. Solid tumors are more likely to do so, however. Of 14 tumors with metastases (excluding the chorionepitheliomas), 9 were in the solid group, 5 in the cystic.

10. In the 19 cases with metastases, the latter were of chorionepithelioma in 5, carcinoma in 5 (adenocarcinoma 4, unspecified 1), multiple components in 3, two components in 1, lymphosarcoma in 1, sarcoma in 1, unspecified histology in 3.

11. Metastasis from the testis must be considered in all instances of supposed primary mediastinal tumor and thorough examination made of that organ, preferably by many serial sections.

12. Of the 4 cases in which the histology of the testes is described, all showed interstitial cell hyperplasia and 3 showed tubular atrophy of moderate or marked extent. In 2 of them, chorionepithelioma was predominant, in 1 it was totally lacking.

13. Confirmation that these changes are all or in part due to one or more hormones secreted by the tumor must await clear identification of these hormones and experimental demonstration of their effect on the testis.

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## DESCRIPTION OF PLATES

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### PLATE 40

FIG. 1. Primary mediastinal tumor, anterior aspect, measuring 18 by 14 by 12 cm. and weighing 1590 gm.

FIG. 2. Cut surface of primary tumor, following fixation, showing the heterogeneous structure with many small cysts, while for practical purposes the tumor is essentially solid throughout. The largest cyst (at the left) is lined with ciliated stratified epithelium of the respiratory type. The nodule at the bottom is deeply hemorrhagic but contains no chorionepitheliomatous tissue.



I



2

Houghton

Malignant Teratoma of Mediastinum

PLATE 41

FIG. 3. Testis. Representative field illustrating the diffuse nature of the interstitial cell hyperplasia and complete atrophy of all but two of the tubules, in which the Sertoli cells persist.  $\times 60$ .

FIG. 4. Testis. Nodule of hyperplastic interstitial cells 0.35 mm. in diameter. Many of similar size were present, the largest being 1.5 mm. in diameter.  $\times 90$ .

FIG. 5. Testis. Interstitial cells growing in the lumen of an atrophied tubule.  $\times 150$ .



3



4



5



# THE VISCERAL PATHOLOGY IN SCARLET FEVER AND RELATED STREPTOCOCCUS INFECTIONS \*

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## REVIEW OF LITERATURE

Our interest in the visceral pathology in scarlet fever was aroused by the occurrence of a striking case in an adult presenting severe destructive lesions, especially of the liver. As a result our curiosity was aroused to the point of making a detailed study of the histological pathology found in the material from all of the autopsies at the Willard Parker Hospital performed on patients dying of scarlet fever, or where that diagnosis was seriously considered after autopsy. In addition, a number of cases of streptococcal infections, either primary or secondary, were also utilized for comparative purposes. In this paper we are presenting a study of the changes occurring in the visceral organs. Suppurative lesions are not included. The cardiac lesions will be discussed in a subsequent paper as they seem to merit special detailed attention.

It is known that tissue changes may occur at a distance from the primary angina in scarlet fever. But it is only within recent years that the concept of generalized toxic manifestations in the various organs has been at all appreciated. Such a concept is not widespread and its expression is to be found practically only in the continental literature. No thorough study of the general changes in scarlet fever has appeared in the American literature since Pearce's study of the cases from the Boston City Hospital in 1899. Although he undoubtedly saw the types of lesions that we have found in the larger visceral organs, he treated them as isolated occurrences. He did not suggest that they represented the effect of a toxic agent, as has been recently emphasized by Kuczynski, Huebschmann, Siegmund, Fahr, and others. Descriptions of changes in the heart, kidney and liver, other than simple degenerations, may be found in the litera-

\* Read before the American Association of Pathologists and Bacteriologists, New York City, April 19, 1935.

Received for publication July 11, 1935.

ture for almost 75 years. We believe it is of some interest to trace the development of the knowledge of these changes and to stress certain aspects of their description, the significance of which has been missed until very recently.

Biermer (1860) reported a case of scarlet fever with interstitial infiltration of the kidneys by a lymphocytic type of cell. There was present also a suggestion of portal infiltration of the liver. Wagner (1867) described 2 cases of scarlet fever with cellular infiltration in the interacinous connective tissue of the liver. Coats (1874) presented a case of interstitial nephritis in the course of scarlet fever.\* In 1877 Klein presented a study of the first series of cases. In 23 autopsies on scarlet fever patients he described renal lesions. The earlier changes he described as occurring in the form of cellular hyperplasia of the capsular epithelium and hyaline degeneration of the elastic intima of the afferent arteries, and also degeneration of the tubular epithelium. The later changes, found in all cases where the individual died after the 9th or 10th day, he described as being an interstitial infiltration by round cells, starting as a slight infiltration around the large vascular trunks. In 8 cases he also studied the liver and found an acute interstitial hepatitis characterized by infiltration by round cells, seen as early as the 2nd day. In 1878 Litten presented a case of postscarlatinal glomerulonephritis with complicating interstitial infiltration in the kidneys. Waller (1880) held that interstitial foci of round cells were common in the kidneys of patients with scarlet fever. He described the lesions as occurring around many of the veins and arterioles of the kidney. The same year Wagner described both the glomerular and the interstitial types of nephritis in scarlet fever. Leyden (1881), although holding that the typical kidney lesion in scarlet fever and diphtheria is a parenchymatous one, agreed that foci of small round cells are frequently found between the tubules and glomeruli. In 1883 Friedländer classified scarlatinal nephritis in three forms: (1) an initial catarrhal stage, occurring during the 1st week, of relatively mild degree, characterized by cloudy swelling of the epithelium of the convoluted tubules, with neither glomerular nor interstitial changes; (2) an interstitial septic form, occurring during the 1st week, but

\* Councilman (1898) cites Litzerich (1871) and Kelsch (1874) as describing interstitial nephritis in the course of scarlet fever. We have not been able to locate the papers.

also seen in the 2nd and 3rd weeks, and characterized by the appearance of round cells in the interstitial tissue (this form is associated with severe throat lesions and was seen in 12 out of 229 cases); and (3) a glomerulonephritis, which he considered the true postscarlatinal nephritis, occurring in the 3rd week, and which he encountered in 42 of 229 cases.

The same year Fischl described periarteritic changes in the small renal vessels in scarlatinal nephritis, and Dunin described foci of round cells between kidney capillaries and tubules. Langhans (1885) described a slight degree of cellular infiltration in the walls of the small renal veins. Crooke (1885), in discussing 30 scarlet fever autopsies, described an interstitial hepatitis, as well as the interstitial lesion of the kidney. The latter, he said, was seldom to be seen during the 1st week of the disease. Nauwerck (1886) described an interstitial infiltration in the kidneys of patients dying of diphtheria, as well as of scarlet fever, as did also von Kahlden (1892). Oertel (1887) described foci of cellular infiltration in the walls of the renal arteries and veins. Sorenson (1891) described glomerulonephritis as the typical lesion of scarlet fever nephritis, but only rarely saw the interstitial variety. Baginsky and Stamm (1893) described interstitial infiltration in the kidneys in scarlet fever with lesions about the cortical vessels. Aufrecht (1893) described round cell infiltration in the liver and spleen, as well as in the kidney. Turner (1894) published a study of scarlet fever nephritis, agreeing in all essentials with Friedländer's earlier description and classification. In 1898 Councilman published his now classic paper in which he described the lesion of acute interstitial nephritis, which he found to occur in about equal incidence in scarlet fever and diphtheria, as well as more rarely in certain of the other infectious diseases. The interstitial foci of round cells (largely plasma cells, and believed by Councilman to be derived from lymphocytes) were found: (1) in the boundary zones of the pyramids, (2) in the subcapsular cortex, and (3) around the glomeruli. A number of cases showed numbers of cells about the long veins of the boundary zone, without infiltration of the interstitial tissue. He minimized the significance of this and did not include these cases in his statistical analysis. The following year Pearce (1899) published a study of the pathology of scarlet fever in which he reaffirmed Councilman's findings with regard to the kidney. In the liver, in 17 of 22 cases, he



found cellular infiltration in the portal areas. In the spleen, he reported endothelial proliferation, but he also noted a lesion which is of considerable interest. In the veins of the spleen, he described a collection of large numbers of lymphoid and plasma cells beneath the endothelium, separating the endothelial layer from the underlying tissues. In some cases this caused irregular projections into the lumen, and in the smaller veins frequently caused almost complete obstruction. In 1900 Roger and Garnier described, in scarlatina, an accumulation of round cells in the connective tissue of the portal areas of the liver, at times almost obscuring vein, artery and bile ducts. The same year Marcuse (1900) studied 114 livers from cases of infectious disease, and a large number showed "lymphomata" in the interstitial tissue. Reichel (1905), in a study of 58 kidneys from cases of scarlet fever, held that the interstitial lesion was characteristic of the early stage of the disease. In 1906 Chapman studied the histological changes in the kidney in scarlatina. In cases where death occurred from clinical nephritis he found typical glomerular lesions with associated tubular degeneration. Those cases where death occurred without clinical nephritis could be divided into two groups. The acute, with death within a few days of onset, showed relatively slight kidney lesions, there occurring slight capsular changes and dilatation of blood vessels with, rarely, thrombi. Severe cases, with death after the 6th day, showed tubular degeneration and infiltration of the interstitial tissue with small round cells. In the earlier cases this was found in the boundary zone of the pyramidal portions and bore some relation to the intertubular arteries. Bingel (1907), studying the liver from patients dying following scarlet fever, described portal infiltration, and in 1 late case, proliferating fibrous tissue. Rach (1909) described the histology of the skin lesion. Thomas (1911), in a study of the adrenal in infectious disease, in an autopsy report of a single case of scarlet fever described an infiltration by lymphocytes, plasma cells and transition forms in the medulla. His more general findings were vascular congestion, edema and granular degeneration of the cortical cells. Schridde (1913) described the interstitial lymphocytic exudate in the kidneys from scarlet fever and diphtheria, and contended that these cells came from the circulating blood, for he described accumulations of these cells in the kidney capillaries several days before the exudation occurred. Landsteiner (1916) described several

cases of interstitial nephritis in scarlet fever in which the principal lesions occurred in the cortex. Munk (1920), in discussing the pathogenesis of the interstitial nephritis of scarlet fever, described infiltration of the liver and adrenals in the same cases. Fahr (1921) stated that in 5 of 8 cases of scarlet fever with myocarditis there occurred an interstitial nephritis. Von Ambrus (1923) studied the pathological histology in 25 cases of scarlet fever. In about half of the cases there was described an interstitial infiltration of the kidneys, either in the vicinity of the capsule, or else in the medulla, chiefly around the veins. Periportal infiltration by lymphocytes in the liver was described. Two years later the same author commented on the frequent appearance of eosinophilic staining cells in the tissues from scarlet fever cases. Herzenberg (1924) attributed the round cell foci, seen in relation to the vessels of the lymph nodes, spleen and liver in the acute infectious diseases, to attempts at extramedullary granulopoiesis. In 1926 Smirnowa-Zamkowa described the pathology of 13 cases of scarlet fever and commented that hyperplasia of the vascular endothelium occurs in all tissues, with appearance of large numbers of mast cells, deemed characteristic, in the tissues. The same year Sysak (1926) reported 32 autopsies on scarlet fever. He described portal infiltration in the liver by lymphocytes, plasma cells, mast cells, and some neutrophilic and eosinophilic polymorphonuclears. In 2 cases the spleen showed plasma cells in the pulp and in the vicinity of the sinus walls. In the kidneys he described an interstitial nephritis. Kahlstorf (1927), in studying the causes of periportal infiltration in the liver, showed that in 36 cases of acute infection 35 showed some degree of portal infiltration. Huebschmann (1929) held that the interstitial infiltration in scarlet fever was a reflection of a toxic process due to the endotoxin of *Streptococcus scarlatinae*. Koch (1930) described a case of scarlatinal nephritis with interstitial foci of round cells. Siegmund (1931) studied 56 autopsies in cases of scarlet fever, paying particular attention to the vascular changes. In addition to the cardiac lesions, among which he described infiltration beneath the intima of the thebesian vessels, he described lesions in the walls of the splenic veins, the small veins of the kidney and liver, the vasa vasorum of the aorta, and also a periphlebitis in the deep peritonsillar tissues. Schottmüller (1931) described a clinical case, with autopsy, of what he termed specific toxic hepatitis and cholecystitis in scarlet fever,

and Fahr (1931) discussed the pathology of the same case. The kidneys showed an interstitial nephritis. The liver showed portal infiltration with involvement of the vein walls. They postulated a specific bacterial toxin. Kettler (1933), in a study of round cell infiltration of the periportal tissue in the liver, concluded that such lymphocytic infiltration occurred most frequently in conjunction with an infectious splenic enlargement, or somewhat less frequently with an interstitial nephritis, and represented the inflammatory result of bacterial toxin. Stein (1933) described a number of cases of scarlet fever with hemorrhage from the great vessels of the neck, both arteries and veins, and in microscopic studies found infiltrative and thrombotic lesions.

In reviewing the literature it will be noted we have attempted to focus attention on the vascular nature of the injury, and the relation of the lesions to the vascular elements of the organs affected. In our own studies we have been impressed with the frequency with which this almost characteristic type of lesion occurs in the various organs from individuals dying of scarlet fever.

The exact nature of the lesion as it appears in different organs varies, depending on the structure of the organ in question. But wherever it does occur it is related to the vascular components of the organ. The lesion, as such, is an interstitial one consisting of an exudate mainly of round cells. These are not entirely of one type. Lymphocytes predominate. Plasma cells are numerous. Other types of less easily classified round cells occur in considerable numbers. Giant cells are practically absent. Polymorphonuclear leukocytes occur, but in small numbers, and but few of these are eosinophiles. Extensive lesions of this nature, which may be considered the indubitable immediate cause of death, have not been frequent, but the less extensive lesions are common. They have been seen deep within the muscular and connective tissues of the pharynx and larynx, in regional and distant lymph nodes, in the lungs, in the pancreas, the testis and the pituitary gland, but rather more commonly in the adrenal, spleen, kidneys and liver.

The statistical analysis of our material is based on 44 cases of scarlet fever plus 15 cases of possible scarlet fever. The material available for study consists of all of the routine autopsy sections. These were, for the most part, Zenker-fixed and stained with phloxine-methylene blue, which is the routine laboratory stain. This

enabled a convincing exclusion of bacterial invasion of the tissues as the cause of the lesions to be described, for where bacteria occur they stand out in bright contrast.

In Tables I and II are tabulated the incidence of the lesions found in the major organs of 44 cases of scarlet fever and 15 cases of possible scarlet fever. The lesions here classified are solely the infiltrative ones; cases or organs called negative may, and usually do, show severe toxic degenerations, as will be de-

TABLE I

*Incidence of Lesions in Principal Organs in 44 Cases of Scarlet Fever*

Organ	Total examined	Grade of severity of lesion				
		0	+	++	+++	++++
Liver .....	44	1	21	12	7	3
Kidney .....	44	12	15	7	6	4
Adrenal .....	38	5	21	9	3	0
Spleen .....	44	9	21	12	2	0

TABLE II

*Incidence of Lesions in Principal Organs in 15 Cases of Questionable Scarlet Fever*

Organ	Total examined	Grade of severity of lesion				
		0	+	++	+++	++++
Liver .....	14	1	8	4	1	0
Kidney .....	14	7	4	1	2	0
Spleen .....	10	2	7	1	0	0
Adrenal .....	11	0	6	5	0	0

scribed below. The severity of the infiltration is indicated by a scale of "pluses," the basis for which is explained below. However, it should be here pointed out that somewhat different scales had to be used for different organs. The most severe lesions found in the spleen and adrenals were in no cases as marked as the most severe seen in the liver and kidneys, and for that reason were not given a scale rating of 4 plus (++++).

In Tables III and IV, for the same cases, there is listed a more complete analysis of each case. The age given is that of the last birthday. The day of illness is taken from the admission histories, and in a few cases is only approximate. The

TABLE III  
Analysis of Clinical, Bacteriological and Pathological Findings in 44 Cases of Scarlet Fever

Autopsy No.	Hours post-mortem	Sex	Age	Day of illness	Blood culture		Microscopic lesions				Remarks
					Ante mortem	Postmortem	Liver	Kidney	Spleen	Adrenal	
436.....	2	M	yrs. 54	9	+	+	1	2	1	..	Acute arthritis
467.....	24±	F	8	7	+	..	1	0	1	..	Clinical acute nephritis
474.....	11	M	5	25	+	..	2	2	2	..	
486.....	..±	F	34	7	..	..	2	4	0	1	Ethmoiditis, meningitis
491.....	24±	M	20	28	..	..	1	0	1	1	Pericarditis
496.....	24±	M	9	3	..	..	2	0	..	3	Empyema
546.....	24±	F	3	6	..	..	3	0	..	0	
559.....	24±	M	29	3	Gram + C.†	..	0	0	3	1	Empyema, mediastinitis
616.....	10	M	2	19	+	+ S. vir.*	1	1	0	1	Adrenal hemorrhage
626.....	12±	M	3	15	..	..	2	1	1	1	
627.....	12±	M	2	30	..	..	1	1	0	1	
646.....	17	F	22	4	..	..	1	0	2	2	Jaundice
647.....	12	M	3	13	..	..	1	0	2	2	Acute pleuritis, peritonitis, chronic endocarditis
647.....	12	M	24	8	..	+ S. vir.*	3	1	0	..	Anaphylactic-adrenal hemorrhage
650.....	14	M	24	8	..	..	2	1	0	..	
659.....	12	M	16	21	..	..	2	1	0	1	
666.....	2	F	2	20	+	+	2	1	0	..	Retropharyngeal abscess
675.....	17	M	20	4	..	+	3	1	0	..	Mastoiditis, meningitis
690.....	4	F	3	20	..	..	1	0	0	1	Peritonitis, empyema
847.....	18±	F	6	48	+	..	1	1	2	1	Peritonitis
855.....	3	F	4	33	+	+ S. vir.*	1	1	..	0	
859.....	6	M	4	18	+	..	1	1	0	2	
863.....	20	M	12	4	..	..	2	1	1	1	Jaundice, edema
866.....	10	M	7	7	Gram + C.†	+	1	0	1	1	? Status lymphaticus
870.....	24±	M	8	28	+	..	1	0	1	2	Acute mitral endocarditis
872.....	24±	M	2	3	..	..	1	2	1	1	
876.....	7½	F	2	18	..	..	2	3	1	1	
877.....	1	M	2	16	..	..	2	3	2	2	Jaundice, surg. scarlet fever
884.....	5	M	5	7	..	..	3	1	2	2	Mastoiditis
888.....	18	M	37	8	..	..	4	3	1	2	Meningitis
889.....	14½	M	5	11	..	..	1	2	1	2	Jaundice
899.....	1½	F	2	23	..	..	3	3	1	1	Mastoiditis, meningitis
902.....	3	M	34	7	..	Diphth.†	1	3	1	3	Mongolian idiot
905.....	13½	F	37	23	+	+	2	4	..	1	Puerperal scarlet fever
957.....	17	F	3	16	+	..	1	0	1	1	
964.....	13	F	22	6	+	..	4	0	2	1	
1069.....	2	F	25	4	..	..	1	0	2	1	Serum anaphylaxis
1077.....	12±	M	3	4	..	..	1	0	2	1	Varicella
1079.....	24±	F	5	3	..	..	2	4	1	1	Mastoiditis, meningitis
1085.....	18	F	4	10	..	..	2	0	0	2	
1091.....	11½	F	6	39	..	..	1	4	0	1	Mastoid
1092.....	9	F	21	4	..	..	2	1	2	2	
1097.....	28	F	3	9	..	..	2	1	3	1	
1102.....	7	F	2	27	..	..	4	1	3	2	
1172.....	..	M	47	10	..	..	3	1	2	3	

† Diphtheroid.

† Gram-positive cocci.

\* Streptococcus viridans.

TABLE IV

## Analysis of Clinical, Bacteriological and Pathological Findings in 15 Cases of Questionable Scarlet Fever

Autopsy No.	Hours post-mortem	Sex	Age	Day of illness	Blood culture		Microscopic lesions				Remarks
					Ante mortem	Postmortem	Liver	Kidney	Spleen	Adrenal	
434.....	24—	F	yrs. 14	7	..	..	—	0	0	..	Previous history of scarlet fever. Non-scarlet fever streptococci recovered from peritoneum
564.....	24—	M	5	50	..	—	0	0	0	..	? Scarlet fever or measles 7 weeks before admission
650.....	14	M	8	51	+	+	2	0	..	..	? Scarlet fever, mastoiditis, meningitis
662.....	12	M	7	14	..	+	1	0	1	1	? Measles or scarlet
687.....	1	M	1	1	..	..	1	0	1	1	19 months old infant with scarlatinaform eruption, dead 18 hrs.
691.....	20	F	3	14	..	+	1	3	1	2	? Scarlet fever 6 days before admission.
842.....	12—	M	1	3	..	—	1	0	1	1	? Measles 3-4 weeks before
878.....	12	M	23	9	..	+ S. alb.*	1	1	1	1	? Scarlet fever. Died few hours after admission
904.....	..	F	13	4 (?)	..	—	1	0	1	1	Probably scarlet fever
981.....	24—	F	30	17	+	+	2	3	..	2	Scarlatinaform rash on 13th day of vari-cella. Died after 3 days
985.....	12—	F	11	..	..	..	2	1	..	..	? Scarlet fever
987.....	15	F	25	6	..	+	2	..	..	2	? Scarlet fever
1062.....	1½	F	2	15	..	S. vir.† Pneum. XIV†	1	1	1	1	Developed scarlatinaform rash on 6th day of sore throat and died
1084.....	2	F	13	5	..	—	3	2	2	2	Scarlatinaform rash 2 weeks after onset of typical pneumonia
1164.....	3	F	6	..	..	B. subt.§ S. alb.*	..	1	..	2	? Surgical scarlet fever following operation
											? Surgical scarlet fever following burn and definite exposure

\* *Staphylococcus albus*.† *Streptococcus viridans*.‡ *Pneumococcus* Type XIV.§ *B. subtilis*.

hour of autopsy refers to the number of hours after death, and is given to serve as a check on the interpretation of postmortem bacteriological studies. In those protocols in which the exact time of autopsy was not stated the symbols  $12 \pm$  or  $24 \pm$  are used, indicating respectively that the autopsy was done the same day on which the death occurred, or the following day (date). The plus (+) and minus (-) blood cultures indicate the presence or absence of hemolytic streptococci in the ante mortem and postmortem blood cultures. Where other or additional organisms were recovered, it is so indicated. In a few of the early autopsies, and in the few autopsies performed after this study was commenced, further bacteriological studies were carried out. These are listed in Table V. For the agglutination-absorption studies we are indebted to Miss Caroline Gurley of the New York City Department of Health Laboratories.

### HISTOPATHOLOGICAL FINDINGS

*Heart:* As mentioned above, we are reserving for a separate presentation the description and discussion of the cardiac lesions in detail. Suffice it to say, at this time, that lesions of varying severity occur in over 90 per cent of the hearts. These fall, more or less, into three overlapping types. In all, the chief cell is some form of round cell. The three types are: (1) an either focal or diffuse interstitial infiltration of the myocardium, having no apparent distribution with reference to the cardiac blood vessels. This type is usually seen in conjunction with either of the following two types. (2) An infiltration either in or about the smaller coronary arteries, which takes the form of an arteritis or periarteritis in which the invading cells are mononuclear, although in some cases there occurs a slight admixture of neutrophilic polymorphonuclears, and rarely eosinophiles; and (3) the commonest type, consisting of a subendothelial infiltration, which may occur beneath the endothelium of coronary veins or beneath the endocardium, more commonly of the ventricular chambers, but which is most strikingly encountered in the walls of the thebesian vessels. This last has been well depicted by Siegmund (1931).

*Kidney:* We are taking up the kidney next, not because it is the most commonly involved, but because it has received the most attention in the past. Nephritis has long been known by clinicians to be a complication of scarlet fever. Since the studies of Klein (1877) and Friedländer (1883), the pathological distinction between the glomerular and the interstitial types has been made, though a long and still not settled argument has been carried on as to which is the "typical" nephritis of scarlet fever. We do not intend to contribute

TABLE V

## Postmortem Bacteriology

Autopsy No.	Blood	Liver	Spleen	Kidney	Lung	Absorption tests	Remarks
434.....	....	....	....	....	....	Non-scarlatinal	Str. hem. from pleural and peritoneal cavities
436.....	Str. hem.*	....	....	....	....	Scarlatinal	Str. hem. from throat
559.....	Gram + cocci	....	....	....	....	....	
626.....	Str. hem.	....	....	....	....	Not in major scarlet fever group. Not in scarlet fever group IV. Not in erysipelas group	
647.....	Str. hem.	....	....	....	....	Erysipelas C <sub>3</sub>	Str. hem. from larynx, cervical lymph node, arm, scrotum and foot.
656.....	Str. hem.	....	....	....	Str. hem.	....	Str. hem. from bronchus
866.....	Negative	....	....	....	....	....	
902.....	Diphtheroid	....	....	....	....	....	
1069.....	Negative	Negative	Negative	....	....	....	Str. hem. from right mastoid
1077.....	Contaminated	....	Str. hem.	....	....	....	....
1091.....	Str. hem.	Str. hem.	Negative	Negative	....	....	....
1092.....	Str. hem.	Str. hem.	Negative	Str. hem.	....	....	....
1097.....	Str. hem.	Negative	Str. hem.	Str. hem.	....	....	....
1102.....	Str. hem.	Str. hem.	Str. hem.	Str. hem.	....	....	....
1172.....	Str. hem.	....	....	....	Str. hem.	....	....

\* *Streptococcus hemolyticus*.

NOTE:—The absorption tests on the last 4 cases had not been completed at the time of going to press.



to that argument except to say that in our experience a glomerulitis is a relatively rare lesion. Only 1 of our cases shows an acute glomerulitis; this patient presented the clinical findings of an acute nephritis and died on the 28th day of illness. There were 8 others, of the 44 scarlet fever cases, in which some histological suggestion of very early glomerular changes was seen. These changes consist of an appearance of increased cellularity of the glomerular tufts, an edema, and in one or two instances a hyaline appearance, of the supporting stroma of the tuft, and an exudation of protein into the capsular space. In none of these cases were thrombi found, or necrosis of any portion of the tuft, or a cellular exudate within the capsular space, or proliferation of the capsule wall. Perhaps a partial explanation of the comparative rarity of the glomerular type of lesion is that more than three-quarters of these patients died by the 21st day of their illness, before the glomerular type of nephritis ordinarily appears. However, we have not been able to carry out any of the special staining procedures, such as those described by McGregor for detailed glomerular studies.

This absence of glomerular damage of any appreciable extent is of interest in relation to the studies of Lyttle on the urine in a series of 14 cases studied at this hospital. Here he found, by use of the Addis sediment count technique, a definite "micronephritis," evidenced by the presence of red cells, white cells and casts, and an increase in protein, of a transitory nature and of a degree commonly demonstrable by these micromethods.

The interstitial type of lesion, on the other hand, is relatively common. About 40 per cent of the cases show either focal or diffuse lesions, and another 33 per cent show an earlier lesion to be described below. The literature contains numerous references (Leyden, Nauwerck, von Kahlden, Councilman, Schridde, and Munk) to cases of interstitial nephritis complicating infections other than scarlet fever. We have not made a detailed study of all the material from all of the cases of infectious diseases in the files at the Willard Parker Hospital, but we are not aware of a case of interstitial nephritis other than in scarlet fever, except where secondary infection by streptococci has occurred. In the files of the pathological division there are over 1000 autopsies (the greatest proportion from cases of scarlet fever, diphtheria, measles, pertussis, poliomyelitis, and varicella) and interstitial nephritis is listed only in cases of scarlet fever

or cases secondarily infected by streptococci. Tracy Mallory (1929), in discussing a case reported from the Massachusetts General Hospital, said: "The majority of cases (interstitial nephritis) follow scarlet fever, though cases do occur in diphtheria, erysipelas, and any acute diseases such as measles in which septic, particularly streptococcic, complications develop."

Typically, the kidney in scarlet fever is both congested and edematous. The congestion is more apt to be medullary than cortical. In rare instances petechial hemorrhages may occur. The glomeruli are more often than not congested, with the capillary tufts distended with red blood cells. It is rather striking to note the relative scarcity of leukocytes within the glomerular capillaries, as compared with the numbers seen in the remainder of the cortical and medullary vessels. The tubular epithelium is almost always the seat of rather marked degenerative changes. Granular degeneration is common and tends to occur rather diffusely. Some degree of desquamation is not uncommon, but casts of the tubules are rare, though in a few instances basophilic amorphous casts are seen. A mononuclear exudate occurs in slightly more than 70 per cent of the cases.

This mononuclear nephritis, which is the lesion described under that name by Councilman (1898), we have subdivided into four groups, based on the degree of involvement of the organ. Schridde (1913) has suggested that there is a pre-infiltrative lesion in which cells of the type that will appear in the interstitial exudate can be found in large numbers in the tubular capillaries. This he described as occurring 2 to 3 days before the appearance of the typical lesion. We have seen tubular capillaries almost plugged by mononuclear cells, but have not found these particularly in the earlier cases, though we do agree that they represent a pre-infiltrative accumulation of the typical cells of the exudate. The lesion that is the earliest which we are able to recognize occurs not about the tubular capillaries but about the long veins on the boundary zone between cortex and medulla. Here are found accumulations of numbers of cells in the adventitia of the veins, but without any actual invasion into the surrounding interstitial tissue. Councilman (1898) described such changes but felt that they were not associated with the more general pathological changes that he was describing. We feel that these periphlebitic lesions precede the more extensive infiltrations, for the latter very definitely appear to spread from these sites. In

the tables these simple periphlebitic exudates are graded 1 plus (+). In our grade 2 plus (++), we include those organs in which there is a beginning infiltration of the surrounding interstitial tissue. In such organs there are usually numerous foci of involvement, each stemming from one of the small veins. Mononuclear cells of varying type are found between the capillary walls and the tubules. When the lesions become more extensive we grade them as 3 plus (+++). Secondary lesions then are usually found in the cortex, particularly immediately beneath the capsule. Invasion of the glomeruli may be said practically never to occur. Secondary foci are also seen in the loose connective tissue stroma just within the pelvic epithelium. These tend to focus about the small dilated capillaries. In a few cases the infiltration becomes entirely diffuse, and these we have graded as 4 plus (++++). Even here, the infiltrating cells are almost exclusively found in the interstitial tissue, though occasionally a few are found within the tubules. The glomeruli may be completely surrounded but invasion does not occur. In these most extensive cases the picture is practically that of leukemia and the name "lymphomatous nephritis," applied by Biermer (1860), the first to describe the condition, is indeed appropriate.

*Adrenal:* The adrenal, in scarlet fever, is almost uniformly congested. In about half of the cases this congestion is marked. However, in only 2 cases in this series did there occur grossly diagnosable medullary hemorrhage; in a 3rd case petechial hemorrhages were found microscopically. In these cases the hemorrhage may have played a rôle in the immediate cause of death. Granular degeneration of the cortical zones is common and where it occurs is not restricted to any one of the zones. The pathological change that we wish to stress is none of these, but one which is homologous to that described for the kidney. It is an accumulation of cells about the medullary venous sinuses. Slightly more than half of the cases studied show such early lesions. These we have called 1 plus (+). About another third show more extensive lesions, infiltrating the interstitial tissue of the medulla, and in 3 of these cases the infiltration continues to a greater or lesser degree into the cortical zones. The last group, consisting of 3 cases, is graded 3 plus (+++), the others 2 plus (++).

*Liver:* The liver is almost uniformly congested. The congestion is usually central, though in more severely involved organs it becomes

diffuse. There is frequently a marked edema, separating the hepatic cells from the sinusoids and infiltrating the portal tissue. This is frequently so severe as to be a probable major contributing factor in death. Degeneration of the liver cells, of varying degree, is almost a constant finding. This usually takes the form of a vacuolization of the hepatic cells and is more usually central in distribution but, in the more severe cases, involves the entire liver lobule. We have never seen in these cases of scarlet fever the midzonal type of liver degeneration described by Opie (1910). In 1 case the fatty degeneration was entirely portal. In a number of cases nuclear vacuolization of the same type seen in the livers of diabetics was seen, and this was particularly true where the liver was evidently profoundly affected, as evidenced by almost complete disorganization of its normal structure.

But, again, the lesion that we wish to stress is the infiltration of the portal connective tissue. As is well known, infiltration of the portal area is a common incidental finding at a great number of autopsies from a great diversity of conditions. Kahlstorf (1927) found some degree of portal infiltration in slightly more than 60 per cent of cases of chronic disease of the gastro-intestinal tract, while in 36 cases of acute infectious disease he found some degree of portal infiltration. Portal infiltration is a common, and undoubtedly not specific, lesion. It occurs, however, in almost 100 per cent of cases of scarlet fever. This becomes more striking when it is realized that over two-thirds of our cases are children under 15 years of age, in whom chronic gastro-intestinal disease is unlikely as a cause of the portal lesions. We have seen it in 43 of the 44 cases of scarlet fever, and in all but 1 of the 15 cases of suspected scarlet fever. Here, again, we believe that the earliest lesion is a periphlebitis or endophlebitis. In the very extensive cases the relation to the finer anatomical structure of the portal space is lost. In 1 case, where death took place within 4 days of onset, the lesion became so extensive that grossly it gave the appearance of multiple necrotizing tumors; microscopically the picture was that of a granuloma, spreading from the portal areas, undergoing central necrosis. Strikingly, in all of the cases with severe 4 plus (+++++) liver infiltration death took place within the 1st week or, at the latest, on the 8th day. It is in the less severe cases that the relation of the lesion to the portal veins becomes apparent, though this is not nearly so marked as is the relation of the

renal and adrenal lesions to the medullary veins. In some cases the infiltration appears to be in relation to the small portal bile ducts. In this connection should be mentioned the work of Smirnowa-Zamkowa (1926) who, in a study of 13 gall-bladders from cases of scarlet fever, recovered streptococci from 10 and histologically found an outpouring of cells deep within the wall about groups of streptococci. We have examined the gall-bladder in only 1 case, but this was negative. We have not seen, however, streptococci in the portal lesions. The picture is not one that is usually associated with acute reaction to direct bacterial invasion.

*Spleen:* Almost all of the cases showed grossly enlarged, congested spleens. Microscopically there is also congestion and edema. The sinusoids are usually widely distended, but they contain relatively few polymorphonuclear cells. For the most part there is some degree of reticular hyperplasia. In most cases the splenic follicles show either hyperplastic or necrotic centers.

In 1899 Pearce described in the veins of the spleen a collection of large numbers of lymphoid and plasma cells beneath the endothelium, separating the endothelial layer entirely from the underlying tissues. In some cases this caused irregular projections into the vessel lumen, in smaller lumens causing almost complete obliteration. We have seen similar, if not quite as extensive, lesions. In addition we have seen the infiltration spread into the walls of the larger veins and even into the fibrous trabeculae. Accumulations of lymphocytes and plasma cells along the walls of sinusoids are common. Some variety of this lesion occurs in three-quarters of the spleens studied, with more or less marked endothelial involvement of the splenic veins or venules in about one-quarter.

*Pancreas:* The pancreas shows only very rarely a slight infiltration with mononuclear cells in the interacinar tissue. In the cases with very severe liver involvement, including an exudate within the portal bile ducts, a similar exudate may be found in the pancreatic ducts. In a number of cases an early, acute interstitial pancreatitis is present.

*Lymph Nodes:* In the distant lymph nodes there occur a reticular hyperplasia and follicular changes similar to those seen in the spleen. Even in the regional nodes, where acute suppuration has not occurred, the predominating cell in the node is of the mononuclear variety.

*Cervical Region:* In some cases, sections of the deep connective tissue of the neck, or even of the pharyngeal musculature, frequently show perivenous accumulations of mononuclear cells with extension into the surrounding interstitial tissues. Lesions of this type are even occasionally seen almost immediately below the acute pharyngeal lesion, where streptococci and polymorphonuclears abound.

*Thymus:* No perivascular changes are seen. The gland is frequently edematous and congested. In some cases there appears to be some hyperplasia of the reticular elements, in others of the lymphoid. This is approximately what Ssipowsky (1928) found in a study of 30 autopsies.

*Pituitary:* In a few cases, cellular accumulations about the vessel walls in the stalk of the gland occur. No infiltration into the glandular portions of the organ are seen.

*Lungs:* Pneumonia is almost a constant finding at autopsy. It is very rarely of the interstitial type, and only occasionally is the peribronchial infiltration, such as has been described in measles and pertussis, seen in scarlet fever. In a few cases, however, rather striking accumulations of mononuclear cells are seen about the smaller pulmonary vessels.

*Salivary Glands:* In a few cases, perivenous and interstitial infiltrations by mononuclears are seen in the salivary glands.

*Aorta:* In 1 case which showed very striking lesions in almost all of the organs already mentioned, the small capillaries of the adventitia of the aorta showed a marked cuffing with mononuclear cells.

*Testis:* One case showed infiltration about the walls of the small arterioles of the testis.

## DISCUSSION

We have shown that in the major visceral organs in severe scarlet fever there occur pathological changes that we have interpreted to be of a single type. We have held that primarily they occur as changes in the vessel walls, with secondary infiltration into the adjacent interstitial tissues. In our sections we have very rarely seen streptococci present at the site of the lesion, except where they obviously represent postmortem invasion. It seems to us reasonable to assume that these widespread changes, almost always primarily associated with the blood vessels, represent the action of a circulat-

ing toxin. To us it seems probable that the primary action is on the capillary and venous endothelium. This is suggested in part by the edema which is such a frequent concomitant. It is perhaps somewhat surprising that capillary hemorrhage does not occur more frequently.

In a majority of the cases, hemolytic streptococci were recovered either from ante mortem or postmortem blood cultures, and also from a large number of the visceral organs where these were cultured. This latter finding is not surprising when it is remembered that the blood cultures were positive. It should be pointed out, however, that in some cases blood cultures, ante mortem and postmortem, were negative, and in others organ cultures were negative, and in certain of these marked visceral lesions were present. We do not feel, therefore, that the presence of streptococci in the blood stream indicates of necessity that they are the direct cause of the visceral lesions, particularly when they cannot be demonstrated histologically at the site of the lesions. It should be borne in mind that in all of these cases hemolytic streptococci were present in the throat, and frequently in other foci where suppurative lesions were present (e.g. otitis, mastoiditis, empyema, adenitis, and so on).

The interstitial mononuclear lesion, which we have described in the various organs, very obviously cannot be held to be specific for scarlet fever. We have seen it in other infectious diseases, notably in diphtheria and in measles. But in these cases the lesion has been of lesser degree and in every instance there has been an associated streptococcal infection; this may be bacteremia, a streptococcal pneumonia, or a meningitis. Unfortunately our bacteriological studies have not been sufficiently complete to differentiate between scarlatinal and non-scarlatinal strains of streptococci in all cases. In a few instances where this has been done, and where the organism has been found to be non-scarlatinal, typical lesions have nevertheless occurred.

We have suggested, as has been previously suggested by others, that the interstitial nephritis and the other similar lesions in other organs are the result of a circulating toxin. Experimental evidence for this is for the most part lacking. The interstitial type of lesion has been produced in the kidney, according to a number of investigators. In 1896 Morse described an interstitial infiltration in the kidneys of rabbits following the injection of a filtrate from the culture of *Staphylococcus aureus*. In 1925 Theobald Smith described an

interstitial nephritis in calves, associated with *B. coli* infection, due to interference with normal colostrum intake. Nye and Parker (1930) have described vascular and perivascular lesions in several visceral organs produced by the intravenous injection of various killed bacteria. Kuczynski (1924) claims to have produced the interstitial lesions of scarlet fever in the liver and kidneys of guinea pigs by the intravenous injection of living streptococci, and except in one instance was unable to recover the organisms on culture. He holds that the lesions were due to the action of an endotoxin. Glomerular types of lesions have been produced by a number of investigators, using a variety of agents. Any interpretation of such results must await a surer knowledge of the substances used in the various experiments, and generalizations should not be attempted without bearing in mind the nature of the lesions as they occur in the course of the normal infection in man. That under certain ill-defined circumstances an interstitial lesion, probably secondary to vascular injury, can be produced in experimental animals by bacteria or their growth products seems fairly certain. That similar lesions may occasionally occur in apparently healthy animals is also probably true.\* The relations of all such lesions to those appearing in the course of naturally occurring acute infectious disease, as in scarlet fever, still await exposition. The frequency of its occurrence in the organs of patients dying of scarlet fever, and the similarity of its appearance in the various organs, suggest the fundamental importance of the lesion in the pathology of the disease.

#### SUMMARY AND CONCLUSIONS

A study of the visceral pathology in scarlet fever and related streptococcus infections is presented. This is based on the histological examination of material from 44 cases of frank scarlet fever, with the addition of 15 cases of presumptive scarlet fever. The literature is reviewed in some detail, covering the last 75 years. These studies reveal the underlying lesion to be one of vascular injury with a concurrent, perivascular round cell infiltration. These changes have been found in from 75 to 95 per cent of the hearts, livers, kidneys, adrenals and spleens of these cases, and to a variable degree in the

\* This has been the experience of the authors, using the mouse as an experimental animal, in an attempt to produce the interstitial lesions.



other viscera, including the pituitary, lung, pancreas, and even the testis. The evidence suggests that these lesions are the result of a circulating toxin. This is borne out by a certain amount of experimental evidence which is reviewed briefly.

NOTE. We wish to express our appreciation to Miss Catherine Gaffney, who carried out much of the bacteriology, and to Miss Caroline Paul, who aided substantially in the photomicrography.

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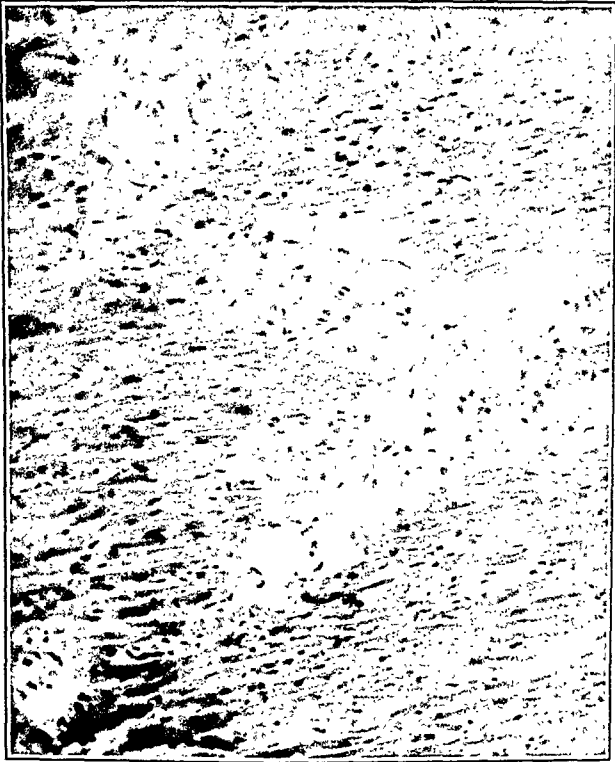
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## DESCRIPTION OF PLATES

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### PLATE 42

- FIG. 1. Heart. Left ventricle, papillary muscle. Grade 1 plus lesion. Note perivascular edema and early round cell infiltration. Minimal changes in heart musculature. About  $\times 150$ .
- FIG. 2. Heart. Auriculoventricular wedge. Grade 3 plus lesion. The outstanding feature is the subintimal infiltration of the thebesian vein, as well as the perivascular reaction. About  $\times 150$ .
- FIG. 3. Heart. High power photomicrograph of blood vessel shown in Fig. 2, illustrating character of round cell infiltration. About  $\times 1200$ .
- FIG. 4. Liver. The perivascular nature of the lesion is best exemplified in a case showing early lesions of a Grade 1 plus intensity. Lesion restricted to portal area. About  $\times 150$ .



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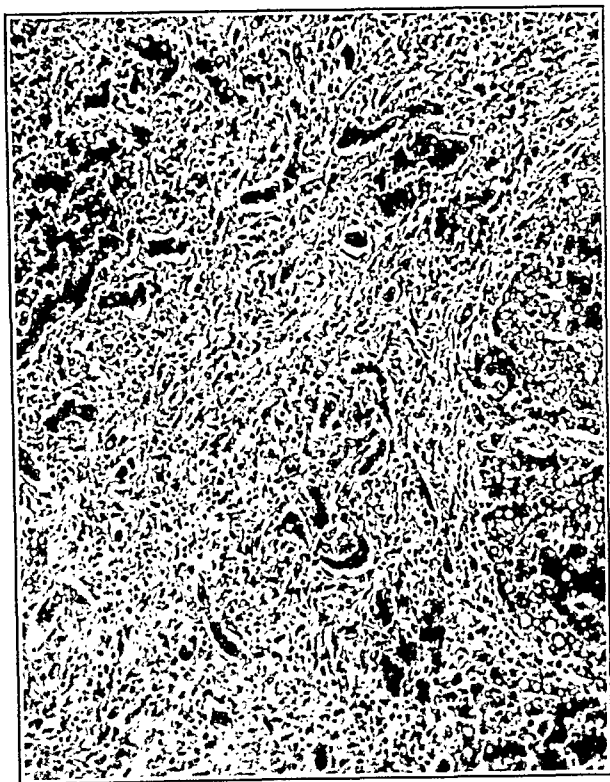
PLATE 43

FIG. 5. Liver. This case presents a severe lesion (Grade 4 plus) with invasion of the liver parenchyma, with bile duct and liver cell regeneration, and severe toxic damage to liver substance.  $\times 150$ .

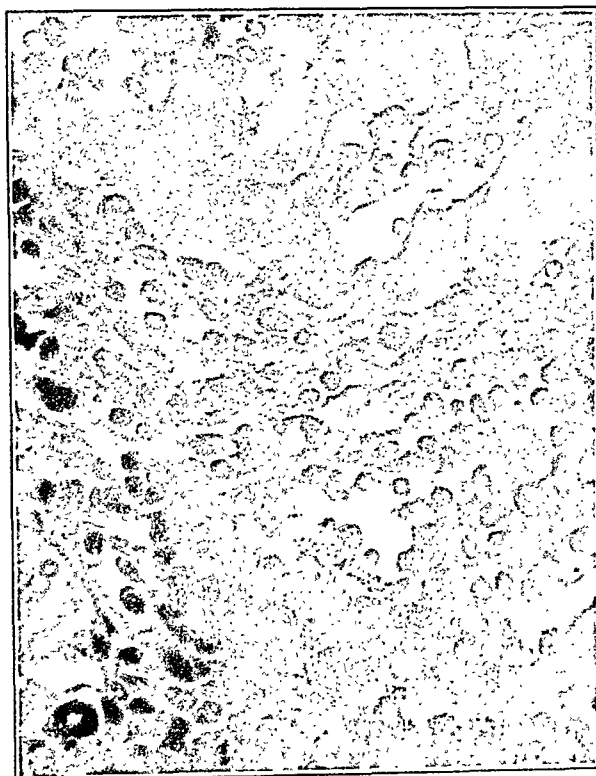
FIG. 6. Liver. High power photomicrograph of Grade 4 plus lesion illustrating vascular origin of lesion and character of the cellular infiltration.  $\times 1200$ .

FIG. 7. Kidney. Illustrating the earliest significant change with the accumulation of mononuclear cells in the distended capillaries of the midzonal region. About  $\times 750$ .

FIG. 8. Kidney. Grade 2 plus lesion showing the subintimal cellular infiltration and extension of the exudate as a perivascular reaction. Note relative absence of glomerular involvement. About  $\times 150$ .



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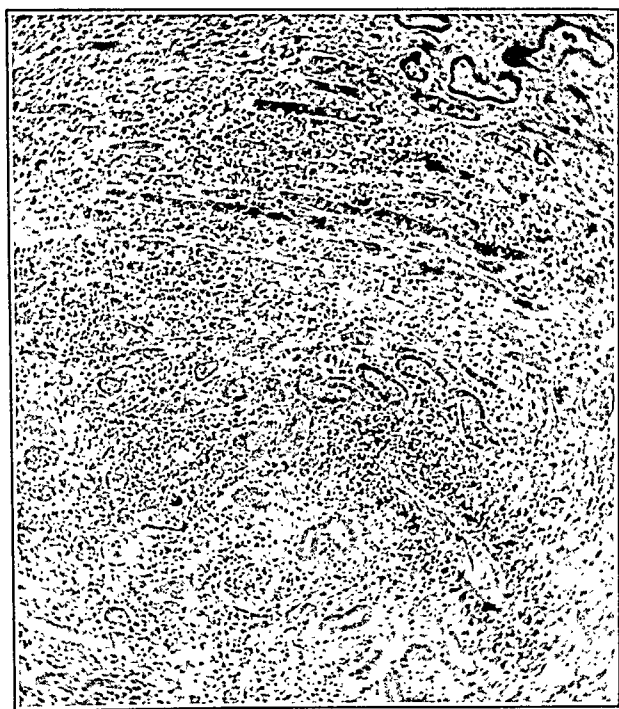
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PLATE 44

- FIG. 9. Kidney. Grade 4 plus type of lesion. The vascular origin is well demonstrated in this instance. Presents a picture almost of the so-called struma lymphomatosa. About  $\times 75$ .
- FIG. 10. Spleen. Grade 2 plus lesion showing much more extensive subintimal mononuclear infiltration, as well as rather diffuse involvement of the splenic pulp. About  $\times 150$ .
- FIG. 11. Adrenal. Grade 2 plus lesion about one of the central medullary vessels and extending into the cortex of the gland. About  $\times 150$ .
- FIG. 12. Pituitary, pars intermedia. Presenting the same type of mononuclear, perivascular infiltration of the pituitary. Lesion most marked in the pars intermedia. About  $\times 150$ .



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## ACUTE AND CHRONIC BACILLARY DYSENTERY \*

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Acute bacillary dysentery may be defined as a systemic disease due to *Bacillus dysenteriae* in which the pathological lesions are caused by one or more toxins. According to this concept the intestine, often regarded as bearing the entire brunt of the disease, is really but one of several organs that may be affected. Evidence will be presented showing that at times extra-enteric manifestations may be far more detrimental and may even dominate the clinical picture.

The intestinal lesions of bacillary dysentery may be reproduced experimentally in rabbits by the intravenous injection of either the living organisms or the toxin. As to the former, we have repeatedly recovered the organism in the intestines under proper control conditions 24 to 48 hours after injection into the marginal ear vein of a rabbit. The blood stream becomes sterile quite rapidly. In the human, positive blood cultures are rarely obtained. We therefore believe that the intestinal lesions in the human are essentially due to the excretion of the dysentery toxin, circulating free in the peripheral blood stream, through the bowel wall into the lumen and so out of the body. Flexner called attention to this important mechanism in explaining the intestinal pathology. There is reason for believing too that in the early stages the toxin may be reabsorbed through the intestinal wall and the cycle again repeated. The process may take place anywhere in the small or large intestine, but there appears to be a special predilection for the distal ileum and the colon where the most advanced lesions are generally found. Olitsky and Kligler<sup>1</sup> have described this thermostabile enteric endotoxin. The same authors isolated a relatively thermolabile exotoxin which has an affinity for the nervous system. We encountered neurotropic strains in 13 per cent of the patients in the Jersey City epidemic<sup>2</sup> and in each case, but none other, there was an associated nasal or

\* Received for publication October 26, 1935.

labial herpes, suggesting an accompanying virus. Spinal fluid examinations were entirely negative, including subdural inoculations into experimental animals. The transitory nature of the meningitic symptoms in these cases suggests rapid reabsorption into the blood stream and excretion through the intestines. In addition to the enteric and neurotropic toxins there appears to be sufficient clinical evidence pointing to the existence of at least two others, the arthritic and myelotropic. The former is suggested by the high incidence of acute arthritis in the acute stage of bacillary dysentery, a condition that may be more troublesome than the diarrhea and which often persists after the diarrhea has ceased. Thus far we have failed to isolate any organism from joint aspirations. In the chronic type of the disease, in which arthritis occurs long after the acute dysentery infection has subsided, the articular or periarticular involvement, which is quite common, is probably due to a streptococcus or streptococcic toxin. The organism is usually the enterococcus which may often be recovered from the intestinal contents, intramural abscesses and catheterized urine specimens of patients suffering from chronic ulcerative colitis. Attention has also been called to the leukopenia frequently encountered in acute bacillary dysentery. In the Sonne-Duval type<sup>3</sup> counts as low as 4000 cells per cmm. were relatively frequent. Within the past year we have seen 3 cases of acute fulminating bacillary dysentery with progressive toxic neutropenia,<sup>4</sup> all terminating fatally within 4 to 5 weeks. The total leukocyte count fell below 1000 cells per cmm., the granulocytes ranging from 2 to 10 per cent and exhibiting varying degrees of toxic change such as swelling of the cell bodies, coarse irregular cytoplasmic granulation with vacuolization, and focal pyknotic areas in the nucleus with partial or complete disintegration. Two of the cases came to autopsy and bone marrow sections showed no evidence of deficiency in granulocyte production. The marked toxic granulocytopenia is therefore due either to the destructive action of the toxin in the peripheral blood or to a necrotizing action on the vascular sinusoids of the bone marrow into which the granulocytes must migrate after their extravascular formation. The term "myelotropic" may therefore be properly reserved for a toxin that affects bone marrow or myelogenic elements.

To summarize, the toxins of *B. dysenteriae* may be considered from the standpoint of selectivity as the enteric, neurotropic, arthri-

tic and myelotropic. They may also be divided according to their mode of action into the excretory (enteric) and absorptive (neurotropic, arthritic and myelotropic). And, finally, according to the degree of toxicity into the toxic non-mannite fermenting strains (Shiga-Kruse) and the comparatively mild mannite fermenting strains (Flexner, Hiss, Sonne-Duval). The last classification is somewhat misleading, however, as not infrequently we have encountered extremely toxic strains in the Sonne-Duval and Mt. Desert types of bacillary dysentery.

The tissue changes incident to infection with *B. dysenteriae* depend largely upon the virulence or selective action of the particular dysentery toxin and the degree of natural or acquired immunity in the host. It appears that some individuals possess a considerable degree of immunity to *B. dysenteriae* infection. This is probably more evident in tropical dysenteric regions than elsewhere, though we believe that this factor is now beginning to play a rôle even in the north temperate zone where bacillary dysentery is prevailing with increasing incidence in many bizarre and hitherto unrecognized forms. A fine example of an acquired immunity was the Jersey City epidemic which gradually waned by the end of August, 1934, only to experience a recrudescence early in September when the non-immunes, chiefly children, returned to the city from their summer vacations. It is increasingly difficult to infect rabbits by the intravenous route once they have been inoculated with even mild strains of *B. dysenteriae*. The permanence of immunity in children or adults is not definitely known, nor can it be judged by a persisting high agglutination titer, but it appears that the period is much longer than is generally supposed.

The earliest intestinal lesion that we have noted in acute bacillary dysentery is a diffuse hyperemia of the mucosa of the small and large intestines with moderate edema and diffuse lymphoid hyperplasia of the solitary acuminate lymph nodules. The latter stand out prominently against a reddened background as grayish, punctate rounded elevations. Peyer's patches are similarly involved, a tuft of dilated blood vessels often surrounding and penetrating the outer margins. Sections taken at this stage reveal lymphoid hyperplasia, edema of the submucosa, plasma cell infiltration and marked congestion of all of the superficial vessels. The goblet cells of the mucosa are actively secreting large amounts of mucus, a process that ap-

pears to be due to irritation and serves as a protective film to the mucosa. At a later stage (the 2nd or 3rd day after the onset of the disease) the acute inflammation of the mucosa becomes more intense, fresh blood appearing to ooze through relatively intact epithelium. Superficial mucosal ulceration occurs, accompanied by hemorrhage due to the action of the toxin on the walls of the submucosal vessels during the process of excretion from the blood stream through the wall of the bowel into the lumen. It appears that the major intestinal pathology is produced within the first 48 hours. The lymph nodules become larger and begin to undergo central necrosis so that portions of intestine fixed in formalin show dimpling of the nodules which often resemble in appearance the mouths of tiny diverticulums. As a general rule the average mild case does not go beyond this stage, which reaches its peak in about 1 week and then gradually recedes, clearing up completely in about 3 weeks. At the end of the 7th to 10th day the diarrhea usually ceases, but this is not synchronous with the subsidence of the pathological lesions. The latter persist, but appear to heal rapidly in uncomplicated cases during the period of obstinate constipation which often follows cessation of the diarrhea. It suggests nature's effort at splinting the bowel in order to favor tissue repair.

The lesions thus far described have been seen through the sigmoidoscope in adults and in autopsy specimens of infants. The colon is involved in every instance, but the process is not necessarily limited to this portion of the bowel. Many cases from the very start are characterized by an acute inflammation of the terminal ileum as well (acute enterocolitis, acute ileocolitis). We have seen 14 such cases in which the clinical features were manifested solely in the right lower abdominal quadrant.<sup>5, 6</sup> Pain and tenderness are present, but no rigidity. Often a mass may be felt in this region and spasm of the sigmoid may be noted in thin patients. There may be a normal or subnormal leukocyte count with a corresponding Schilling picture at the onset of the disease. The pyrexia generally varies from 99 to 101° F. All of the patients referred to were operated on for acute appendicitis, but the appendix was found to be quite normal. The mesentery, however, was studded with numerous large, pinkish, succulent lymph nodes and the distal ileum was intensely reddened, swollen and clearly demarcated from the normal light grayish pink color of the contiguous healthy intestine. The

vascular arborizations were greatly exaggerated and the nodes along the mesenteric border of the ileum, at the ileocecal angle and in the mesocolon, participated in the acute process. In most cases thus far studied the acute distal ileitis appeared to subside in a few weeks, but in some the condition persisted. The association of an upper respiratory infection with mesenteric adenitis was occasionally noted in children, particularly in the Sonne-Duval type of bacillary dysentery. The diagnosis may be made clinically by means of the features above noted and the accompanying diarrhea. Epidemiological investigation often reveals one or more contact cases. The laboratory diagnosis depends on the following triad: fecal culture, diagnostic fecal bacteriophage and agglutination titer. The specific dysentery organism is often isolated only during the 1st week, after which diagnostic bacteriophage appears in the feces and the agglutination titer of the blood begins to rise. Repeated studies for all three should be made on alternate days for at least six examinations. The agglutination set-up should include all strains of *B. dysenteriae*, not omitting the Sonne-Duval organism which is now being detected with increasing frequency. We prefer the macroscopic method, 4 hours incubation at 55° C. followed by ice-box incubation overnight. Smooth, agglutinable strains must be used. It should be noted that diagnostic titers in Sonne-Duval dysentery often do not exceed 1:50 while in all the other types a minimal titer of 1:100 is required.

The acute stage of bacillary dysentery, as we have encountered it (not including Shiga-Kruse type), generally subsides quite completely by the end of the 3rd week. Intestinal symptoms persisting beyond this time indicate chronicity and the probability of ultimate secondary non-specific intramural infection. Sigmoidoscopic examinations of patients with chronic dysentery reveal a boggy reddened mucosa which bleeds upon the slightest trauma by the instrument. Discrete or serpiginous ulcerations of the mucosa may be present and focal areas of lymphoid hyperplasia may be noted if the overlying edematous mucosa does not obscure the picture. The symptomatology includes periodic bouts of cramps, generalized abdominal soreness, and mucoid, watery or bloody diarrhea. These exacerbations may only last 1 to 3 days and in the intervening period there is no special complaint other than an indefinite abdominal discomfort. There appears to be no clear line of demarcation between this phase of the disease clearly traceable to an original

infection with *B. dysenteriae*, the acute infection never having completely cleared up, and non-specific ulcerative colitis. The chronic stage of bacillary dysentery, therefore, blends imperceptibly with the non-specific stage, so called because the original infection has often been lost sight of. The dysentery organism has disappeared and secondary infection, usually with the enterococcus and *B. coli*, takes place through the mucosal ulcerations originally produced by the toxin of *B. dysenteriae*. The denuded areas extend in all directions, become confluent, and extensive serpiginous or geographic mucosal denudations occur. The small remaining islands of intact epithelium are often pinched off and swollen with accumulated secretion, edema and cellular infiltration so that they may appear as pseudopolyps, termed "polyposis cystica" by Virchow. The secondary infection penetrates deeply into the wall of the bowel, along the mural lymphatic pathways, and not infrequently localized abscesses are formed in the submucosa or in the region of the circular intermuscular lymphatic plexus. The intramuscular lymphatics appear to form a natural pathway for spread of the infection. Intramural abscesses may break through the serosa into the peritoneal cavity, but usually a productive inflammation of the peritoneum occurs in advance of the slowly enlarging abscess so that by the time rupture occurs the area is well walled off and a generalized peritonitis fails to occur. The small submucosal abscesses frequently break through the overlying mucosa, if such be present, into the lumen of the bowel and the purulent material is eliminated with the feces. Clinically, intramural abscess formation is accompanied by marked daily rises and remissions of temperature of the septic type, which disappear soon after the abscess breaks through into the bowel lumen with subsequent drainage. Most of these cases go on for years and during this time it is the exceptional patient who succeeds in epithelializing over the extensive ulcerated areas. In an attempt to combat the spread of intramural infection, or possibly as the result of the usual mode of healing where loss of tissue has occurred, extensive intramural fibrosis occurs. This may be seen while the secondary non-specific infection is still active. Sections taken through the wall of the bowel reveal mural thickening five to fifteen times the normal, and fibrous connective tissue replaces most of the coats so that only an occasional fragment of intact circular or longitudinal musculature can be discerned. The wall is

infiltrated with mononuclear cells, plasma cells, polymorphonuclears, and at a relatively early stage marked reticulum cell hyperplasia may be seen. The solitary acuminate lymph nodules disappear and the intestinal wall becomes a thick rigid tube consisting chiefly of vascularized connective tissue. It is this pathology that accounts for the loss of haustration and extreme narrowing of the intestinal lumen, regarded as diagnostic roentgenographical criteria typical of non-specific ulcerative colitis. We have noted also an accompanying hypertrophy of the Meissnerian and Auerbach nerve plexuses in the fibrotic stage of chronic ulcerative colitis. The process above described is not always limited to the colon where it often assumes a segmental distribution clearly delineated from contiguous normal bowel. It may extend beyond the ileocecal valve into the distal portion of the ileum (distal ileitis) where it produces essentially the same picture as in the colon, except that granulomatous giant cells are often encountered and may lead to a mistaken diagnosis of tuberculous ileitis. Search for tubercle bacilli and guinea pig inoculation of the macerated tissue will, however, invariably prove negative. In the ileocecal region a productive type of inflammation with marked mural thickening and stenosis of the bowel may occur ("non-specific granuloma"). The microscopic picture reveals intramural infection, fibrosis and many giant cells. Any other part of the small intestine may be involved, often in a somewhat segmental fashion, giving rise to so-called "skip-areas" of chronic inflammation.

#### SUMMARY AND CONCLUSIONS

The evidence on which the pathogenesis of the lesions that have been described is based may be summarized briefly as follows:

1. Follow up studies of cases of acute bacillary dysentery have shown that in some patients the intestinal lesions persist long after the specific dysentery organism has disappeared from the feces. In these cases the agglutination titer remains high, with the possible exception of Sonne-Duval dysentery in which the initial as well as subsequent titer may remain relatively low (1:50). Diagnostic bacteriophage and positive fecal culture may be demonstrated in some cases. The longest period of observation in this group is 4 years, the shortest 6 months. In the follow up studies of the Jersey City epidemic,<sup>7</sup> 122 out of a total of 210 hospitalized cases were re-



examined 9 to 12 months after the acute attack and 18.8 per cent gave definite clinical or roentgenographical evidence of chronic ulcerative colitis.

2. Epidemiological studies have revealed a definite contact and familial incidence in non-specific ulcerative colitis and a geographic distribution corresponding to that of bacillary dysentery. The contention that non-specific ulcerative colitis cannot be transmitted by contact is only partly true for the disease is actually transmitted in this manner during the period of initial infection with *B. dysenteriae*. We have offered evidence of the familial incidence of non-specific ulcerative colitis, the patients all exhibiting diagnostic titers against *B. dysenteriae* and in addition, in some instances, diagnostic phage.<sup>8</sup>

3. Positive agglutination titers against *B. dysenteriae* have been obtained in 62 consecutive cases of non-specific ulcerative colitis, in 2 cases of chronic non-specific granuloma, and in 14 cases of chronic distal ileitis. Acute distal ileitis has been encountered in 14 cases of acute bacillary dysentery.

4. Control studies<sup>9</sup> of 300 serums showed an incidence of diagnostic titers against *B. dysenteriae* of only 4.6 per cent, as compared with the figures obtained above in non-specific ulcerative colitis.

The facts above presented are perhaps not without their practical significance. The character and mode of action of the dysentery toxins suggest that to be most effective a specific therapeutic serum must be used within the first few days when the major intestinal lesions occur. Moreover, antidysentery serum should be essentially an antitoxin effective against known toxins that have been properly standardized by biological methods. Active immunization must be directed chiefly against the secondary non-specific invaders, for residual dysentery infection is the exception rather than the rule. Surgical excision is not advisable, except in an emergency, as resections are generally made through infected bowel with resulting recurrence of the original condition. Intramural infection often extends well beyond the line of visible demarcation. In some cases "skip-areas" of segmental inflammation are completely overlooked, for they may occur anywhere in the small or large bowel.

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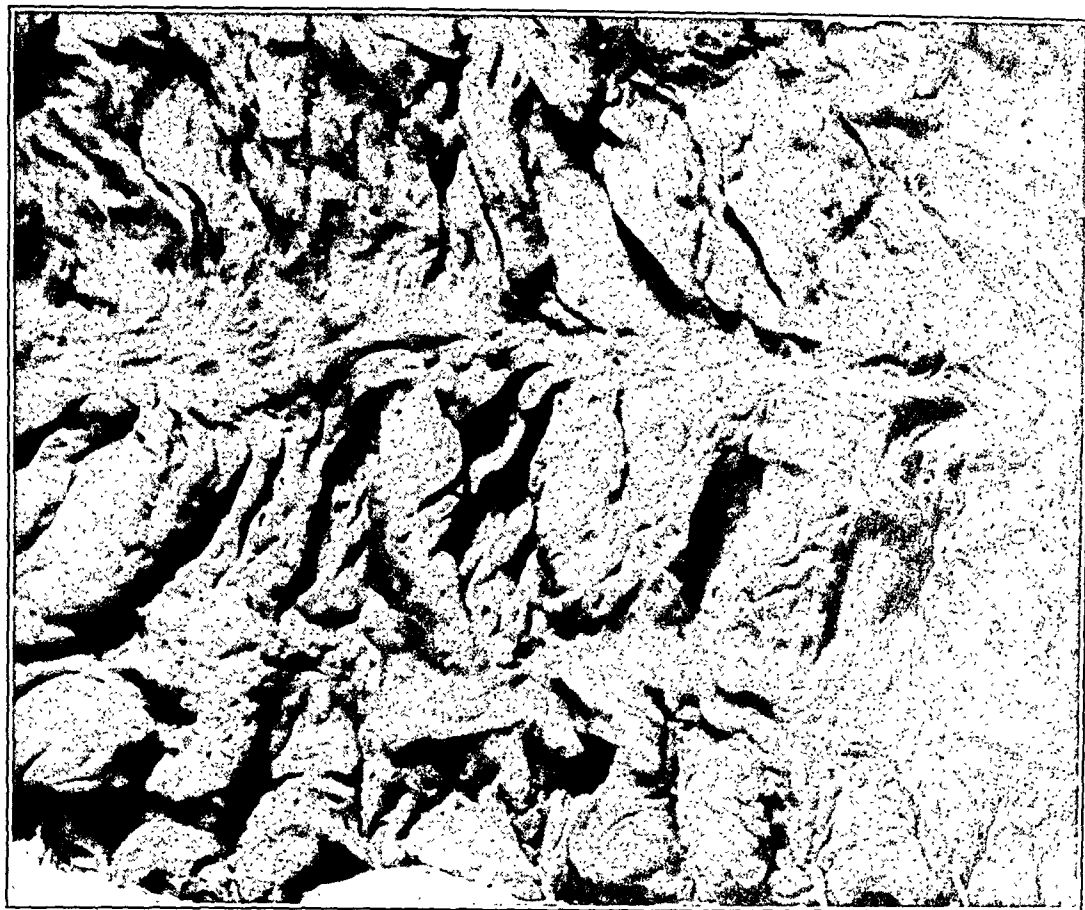
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## DESCRIPTION OF PLATES

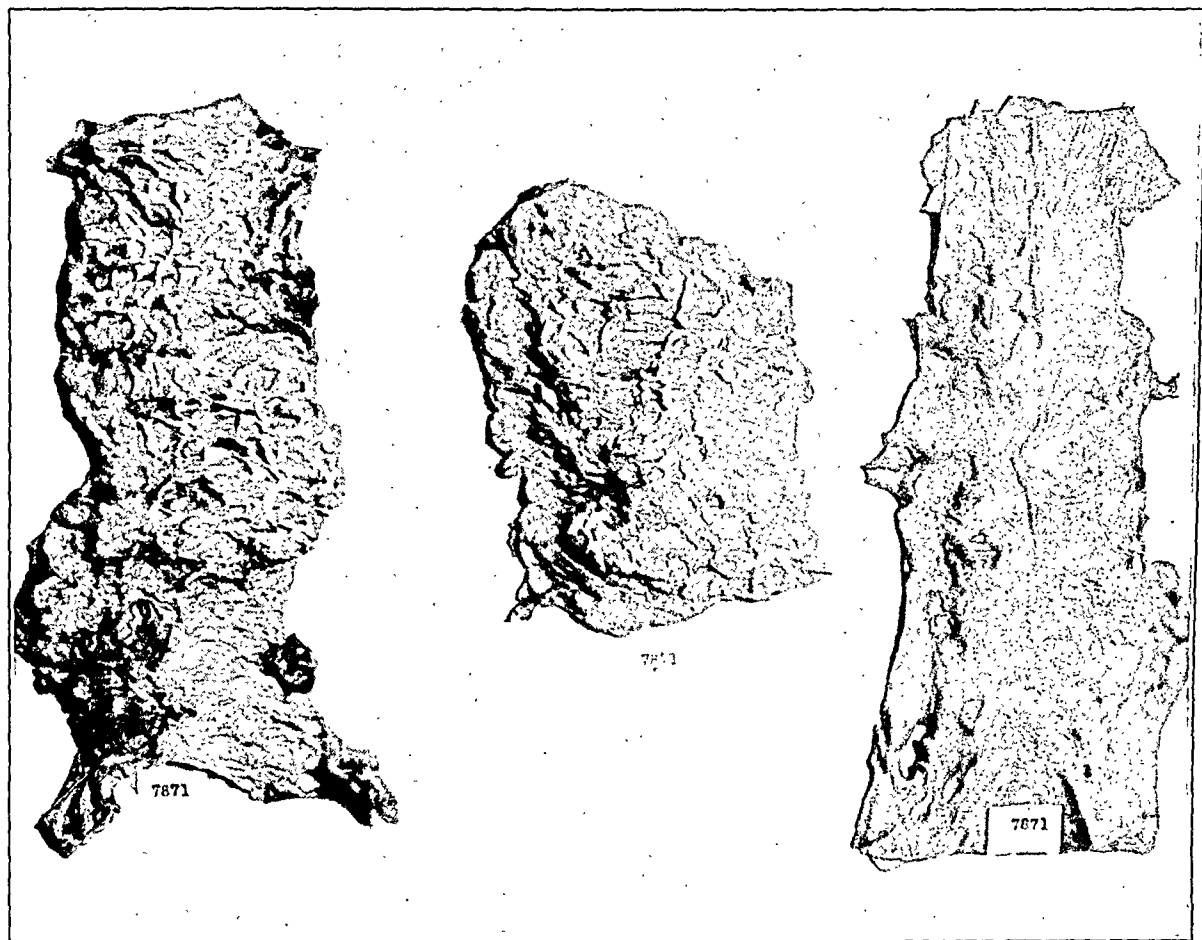
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### PLATE 45

- FIG. 1. Punctate follicular hyperplasia and necrosis seen in early stage of acute bacillary dysentery (Flexner type).
- FIG. 2. Acute fulminating bacillary dysentery (Mt. Desert type) with marked toxic neutropenia. Note advanced ulcerative lesions in bowel. Death followed perforation during the 5th week of the disease.



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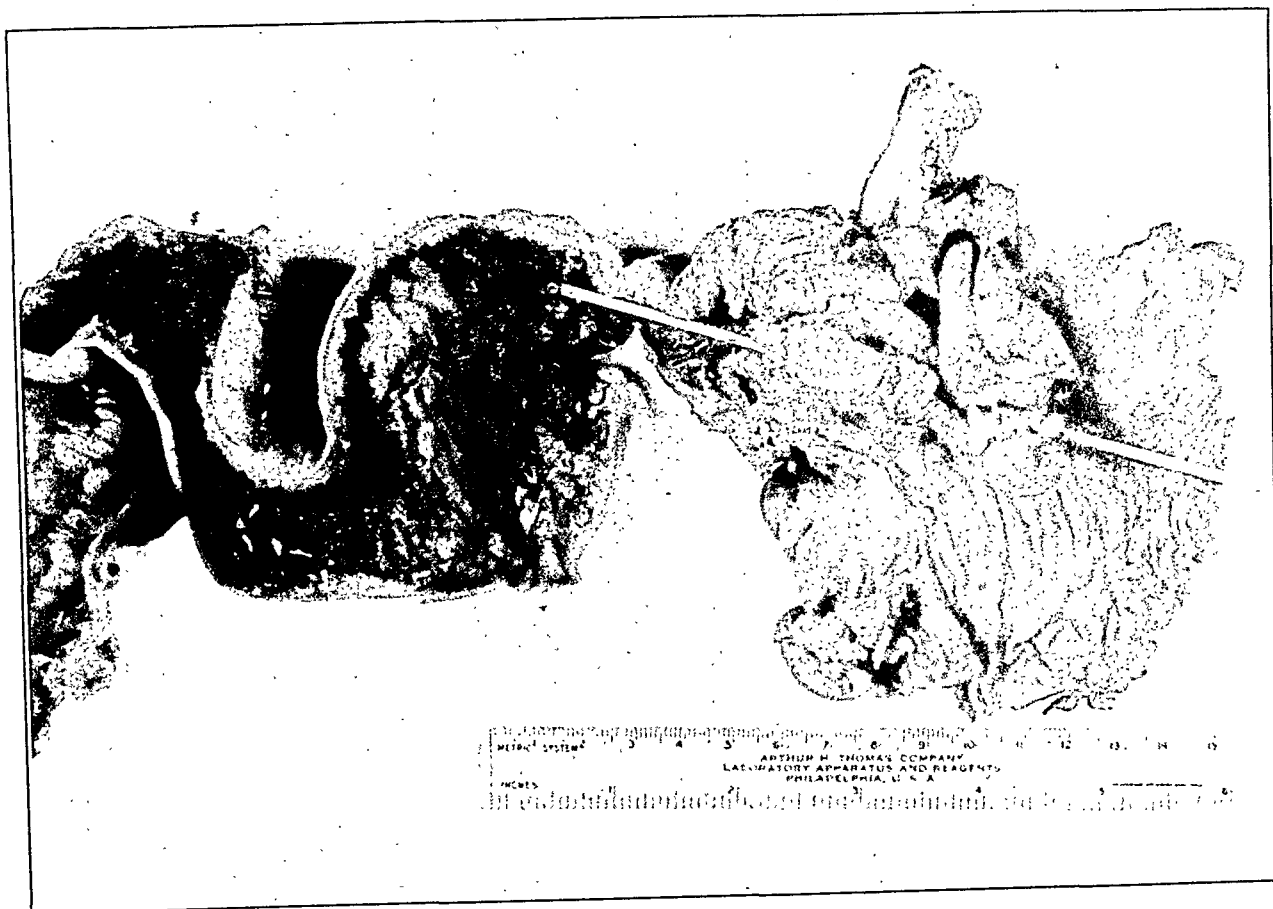
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Felsen

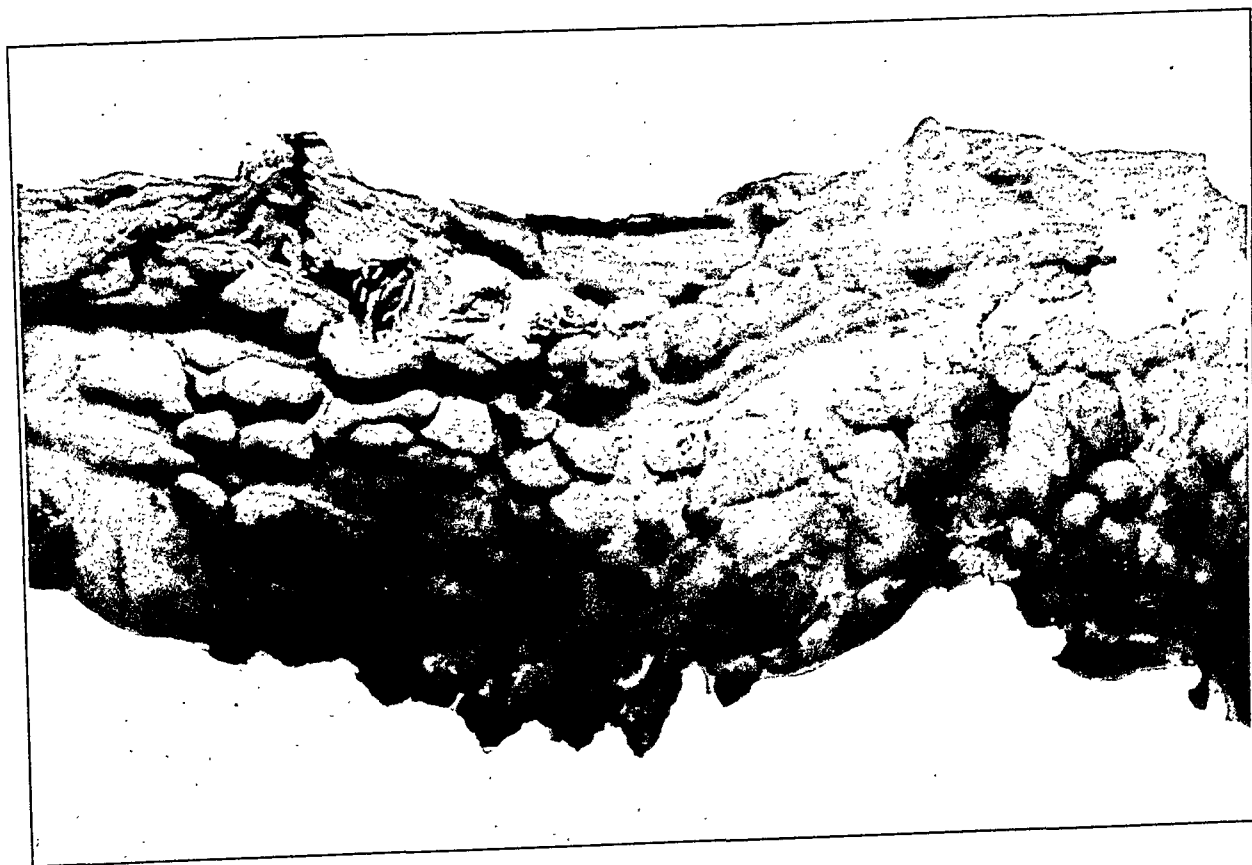
Acute and Chronic Bacillary Dysentery

PLATE 46

- FIG. 3. Resected portion of ileum showing chronic distal ileitis. One year later the patient, a physician, was still excreting *B. dysenteriae Flexner*. This is a productive type of inflammation with giant cells and is often confused with tuberculosis.
- FIG. 4. Chronic ulcerative colitis in a child following infection with *B. dysenteriae*. Note serpiginous ulceration, inflammatory polyposis and mural thickening due to fibrosis.



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Felsen

Acute and Chronic Bacillary Dysentery



## A VIRUS DISEASE OF OWLS \*

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A great horned owl (*Bubo virginianus*) found dead and furnished us for study has yielded information sufficient to establish the existence of a virus disease affecting that species. Circumstances surrounding the finding of this carcass have been described by Errington.<sup>1</sup> The mate of the owl was picked up dead in a highly decomposed state at a later date under the same roost tree. Apparently both birds of this pair succumbed to the infection at about the same time. The disease occurred in the wintertime, the first owl being found freshly dead on Feb. 21, 1932.

The bird received for examination, No. B-10214, was an adult in full flesh, weighing 1134 gm. As the crop contained considerable food, extreme illness was probably not of long duration. The liver was congested and studded with fine necrotic areas. The spleen was enlarged and covered with many small abscesses of varying size. Other organs appeared normal. Cultures yielded a diphtheroid organism which was found non-pathogenic for owls.

Transmissions by means of a pooled suspension of liver and muscle were immediately attempted. A horned owl, No. T-414, injected with liver and muscle suspension, died after 6 days without showing marked symptoms until just previous to death. The spleen was greatly enlarged, consisting of a solid mass of firm abscesses of pin-head size. The liver was covered with necrotic nodules which were not nearly so numerous, however, as in the spleen.

Owl No. T-412, a screech owl (*Otus asio*), injected at the same time, was found dead after 7 days. The spleen did not appear enlarged but showed a few indistinct areas of apparent necrosis. The

\* Presented before the joint session<sup>2</sup> of the American Association of Pathologists and Bacteriologists, and the American Association of Immunologists, New York City, April 18, 1935.

Received for publication October 26, 1935.



liver appeared normal and showed no evidence of necrotic areas even upon close examination. Other organs also appeared normal.

A barred owl (*Strix varia varia*), No. T-413, injected with the original material, remained well. Two guinea pigs and two pigeons inoculated with infective material remained well.

A considerable time elapsed before more owls were available for transmission, and when an attempt was made to produce further experimental infections it gave negative results.

Examination of microscopic sections showed the presence of very definite and peculiar inclusion bodies both in the original owl and in the two experimental owls succumbing to the infection. The presence of these inclusion bodies demonstrated that the infection was caused by a virus and was successfully transmitted to owls experimentally. A description of the microscopic findings follows.

#### MICROSCOPIC EXAMINATION

*Owl No. B-10214:* Horned owl (*Bubo virginianus*) found dead of natural disease. Necrosis of the liver cords was evident throughout the lobe examined, and in certain areas little staining cytoplasm was left. Scattered throughout the liver were numerous well defined necrotic abscesses. These consisted of foci of rather uniform cytoplasmic débris, in the center of which was a concentration of nuclear fragments. Many of these nuclear fragments could be seen to surround partially and to be attached to eosin staining material of a much deeper hue than the cytoplasmic débris. This eosinophilic material appeared to consist of fragments of inclusion bodies. Toward the periphery of the abscess were intact nuclei with no definite relation to cytoplasmic material. The nuclei, in general, were heavy rings entirely filled with a deep staining matrix, reddish purple in color, and represented nuclei entirely filled with inclusion body material. Farther toward the periphery were nuclei definitely associated with masses of necrotic cytoplasm having some resemblance to the original liver cords. These nuclei carried inclusion bodies often well separated from the nuclear membrane. The inclusions varied from small eosinophilic bodies within an almost normal nucleus to masses of deeply stained eosinophilic material completely filling the nuclear ring. In the small necrotic areas there was little evidence of cellular infiltration, the entire amount of nuclear residue appearing

to come from cells normally within the area. The larger abscesses showed definite zonal structure and the center of the abscess was occupied by much more nuclear debris than could originate from cells normally in the area. It was not possible, however, to recognize clearly that this debris originated from infiltrating cells. A few large vesicular nuclei indicated some cellular proliferation within the abscess, which might also account for the concentration of nuclear elements. A definite zone of nuclei containing well defined inclusions surrounded the abscesses, whether large or small. While a few inclusions were found generally scattered throughout the liver, they were usually grouped peripherally with relation to the abscess formation, as shown in Figure 1. Generally those inclusions found somewhat apart from the definite abscess were of the smaller type, not filling the entire nucleus, and appeared to be in the formative stage. In a few areas of the liver there was evidently a definite cellular infiltration limited to rather small areas, but the cells seemed to be independent of either the localized necrosis or the occurrence of inclusion bodies.

*Owl No. T-414:* Horned owl (*Bubo virginianus*) inoculated experimentally. The liver, covered grossly with necrotic areas, presented microscopically a mass of abscesses varying from small to very large. The general structure of the abscesses was similar to that described for the naturally infected owl, except that the focal structure was even more marked. The abscesses were much larger and the inclusions more numerous and more completely developed. In some medium power fields the hundred odd nuclei in evidence all contained uniformly an inclusion body filling the entire nucleus.

A section of the spleen showed this organ to consist of a mass of macroscopic abscesses. Little normal structure of this organ could be made out microscopically because of widespread necrosis and masses of nuclear debris. Numerous nuclei were discerned carrying well defined nuclear inclusions similar to those seen in the liver. Some of the nuclei appeared to be in the reticular cells, but in most cases the type of cell could not be determined. Several well defined inclusions were evident in cells making up the splenic capsule and in cells quite definitely representing the endothelium of blood vessels. A number of capillaries showed proliferation of the endothelium.

A section of kidney taken from this owl showed moderate necrosis of the tubular epithelium. Many of the tubules contained calculi

which obviously were not a part of the infective process. No cellular infiltration, no focal necrosis and no inclusion bodies were present in the kidney.

*Owl No. T-412:* Screech owl (*Otus asio*) inoculated experimentally. Microscopic examination of the liver showed the presence of widespread necrosis. The process was of lesser degree than that seen in the case of the great horned owl, both for the natural and the experimental infection. The liver did not show any dense collections of necrotic debris which could be called an abscess in the usual sense of the term. Cells containing inclusion bodies were, however, scattered throughout the liver tissue, occasionally occurring in somewhat localized groups. These collections of inclusion bodies were typically associated with a small area of advanced necrosis in the liver cord cells. This picture represented without question an earlier stage of the abscesses than that seen in the case of the horned owl. In these early lesions there appeared to be no infiltrating cells whatsoever. Scattered throughout the liver, however, were occasional areas of perivascular infiltration. These lesions did not appear to be associated with any marked necrosis or with the presence of inclusion bodies. Not only were the inclusion bodies in the liver of the screech owl much less numerous than in the case of the horned owls, but most of the inclusion bodies themselves were smaller. For the most part, the inclusion bodies did not fill the entire nucleus and occupied but a small part of the intranuclear space, distinctly separated from the nuclear wall. There were, however, many inclusions identical with those seen in the horned owls.

The spleen, which showed no changes grossly, also appeared normal microscopically. Sections of lung showed a rather marked degree of congestion but the alveolar spaces appeared free of exudate.

## DISCUSSION

The common occurrence of very typical inclusions in all three owls can leave no doubt that the disease is caused by a filterable virus. The consistent location of the inclusion bodies in the liver indicates that this organ is typically involved in the infection. From the extensive damage done to this organ it would seem to be the principal site of the infection. That the spleen may be involved is shown by the definite inclusion bodies in the spleen of one infected owl.

The manner of development of the lesions in the liver may be surmised from a study of the available material. The earliest lesion is apparently cytoplasmic necrosis of a few adjacent cells with the formation of intranuclear inclusion bodies. Cytoplasmic necrosis develops concurrently with the appearance of small inclusion bodies within the nuclei. Often the degenerating cytoplasm shows numerous acidophilic bodies which are much lighter staining than the intranuclear inclusions. These cytoplasmic bodies are shown faintly throughout the cytoplasm in the central cell in Figure 4. As the mass of cells involved increases in size, there appears nuclear fragmentation of the centrally located nuclei. With nuclear fragmentation the outlines of the inclusion body become less distinct, but in many cases the intact inclusion can be observed with attached fragments of chromatin or nuclear membrane. With further development of the lesion the central mass of nuclear fragmentation becomes very dense, as shown in Figure 1. It does not seem possible that all of these nuclear fragments could originate from locally distributed cells. As no intact cellular elements could be made out, it would seem that cells infiltrating into the area quickly became necrotic and underwent fragmentation. With the formation of the dense collection of necrotic material there develops a peripheral extension of the process which results in a wide surrounding zone of nuclei containing well defined inclusions and marked cytoplasmic necrosis. This advancing zone of involvement is often rather sharply demarcated from the normal liver tissue.

An apparent development of the intranuclear inclusions may be followed through various stages of formation. The first small acidophilic body occurs before any great change has taken place in the nucleus. As the inclusion increases in size there is evident degeneration in the interior of the nucleus with progressive margination of chromatin. The cell cytoplasm becomes necrotic. As the inclusion increases in size, margination of chromatin becomes more marked and coarse in structure. Some of these stages are shown in Figure 3. Finally, the inclusion fills out the nucleus and comes into actual contact with the nuclear membrane, so that the nucleus appears as a heavy ring entirely filled with deeply staining acidophilic material. Sections cutting through large masses of marginated chromatin often show a clear space between the inclusion body and the mass of chromatin. This is shown in the central cell in Figure 5.

## SUMMARY

A virus disease has been recognized as a natural disease in the great horned owl (*Bubo virginianus*). The infection has been transmitted experimentally to the same species and to the screech owl (*Otus asio*), but not to the barred owl (*Strix varia varia*). The disease is characterized by the formation of multiple abscesses in the liver and, less consistently, in the spleen. Large intranuclear inclusion bodies occur typically in the hepatic cells.

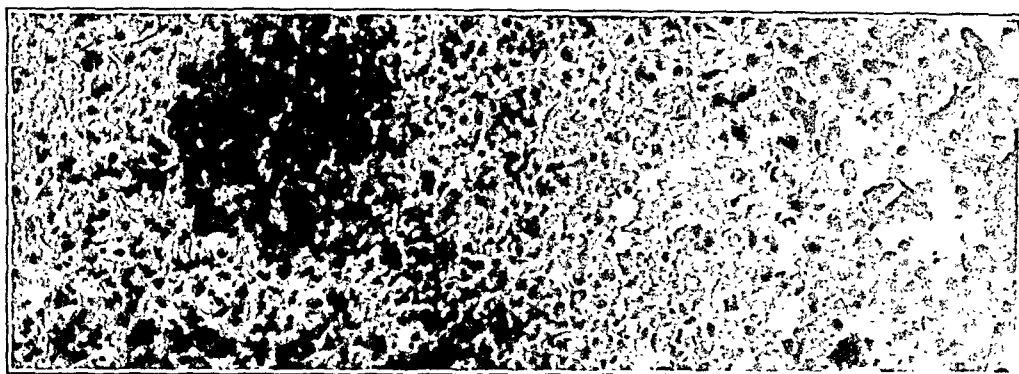
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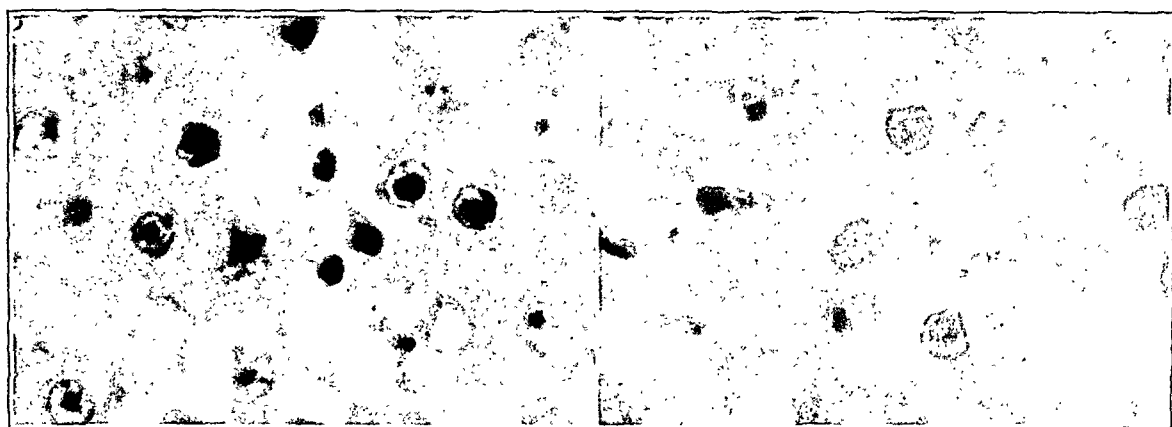
## DESCRIPTION OF PLATE

## PLATE 47

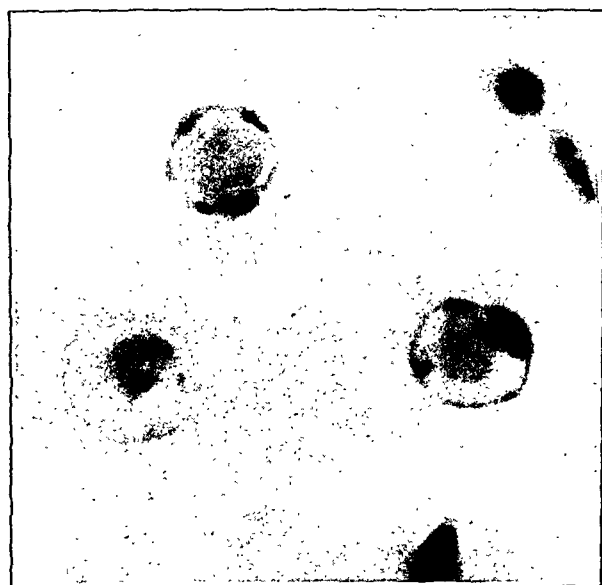
- FIG. 1. Owl No. B-10214. Liver. Miliary abscess, late lesion: cellular infiltration following necrosis; zone of inclusion bodies lies between dense infiltration and normal liver tissue seen on right.  $\times 200$ .
- FIG. 2. Owl No. T-414. Liver. Marked necrosis of hepatic tissue with intranuclear inclusions. Early lesion; no cellular infiltration.  $\times 750$ .
- FIG. 3. Owl No. T-412. Liver. Hepatic nuclei showing margination of chromatin and inclusion bodies in the three stages of development.  $\times 2200$ .
- FIG. 4. Owl No. T-412. Liver. Developing inclusion body in nucleus of hepatic cell. Marked necrosis of cytoplasm with numerous small cytoplasmic bodies.  $\times 2700$ .
- FIG. 5. Owl No. T-414. Liver. Nuclei of hepatic cells showing fully formed inclusions filling the entire nucleus. Chromatin in heavy peripheral masses.  $\times 2200$ .



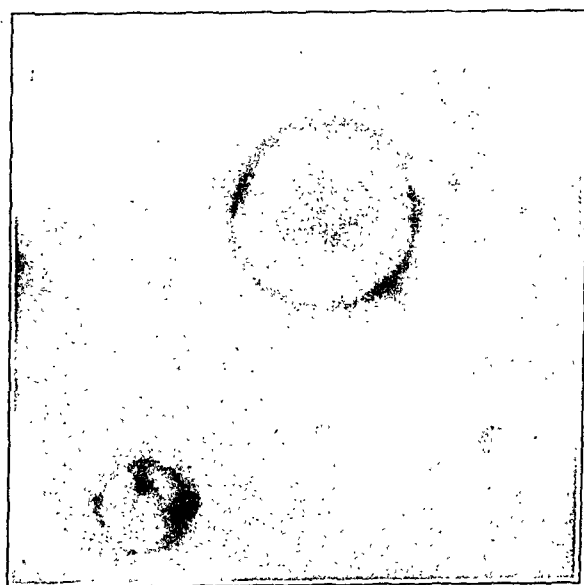
I



2



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5



# SO-CALLED ATROPHY OF THE ADRENAL CORTEX WITH INTRANUCLEAR INCLUSIONS \*

## REPORT OF A CASE

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New Haven, Conn.)*

Since Addison in 1855 first described the symptom-complex now known by his name, it has become common knowledge that by far the largest number of cases are associated with bilateral and extensive tuberculosis of the adrenals. That there are, however, numerous other destructive processes involving these glands and reproducing more or less faithfully the same clinical syndrome also has long been known. Of these, the most interesting and most frequent (about 15 per cent of all reported cases of Addison's disease<sup>1</sup>) is a process resulting in bilateral selective cortical damage. This was recognized as early as 1885 at least, when Coupland<sup>2</sup> reported a case of "simple atrophy" and was able to find 6 more in the English literature. By 1908 Bittorf<sup>3</sup> had collected 47 cases. In 1929 Guttman analyzed 68 reported cases (1900-1929) and the last decade has witnessed an even greater increase in interest in this disease.<sup>1, 4, 5, 6, 7</sup> Our knowledge, however, of the etiology and pathogenesis of this strange entity has hardly kept pace with the accumulation of gross and histological data, and this is mirrored by the multiplicity of names with which it has been designated — chronic idiopathic (primary) suprarenal insufficiency (Bittorf), simple atrophy (Karakascheff), idiopathic atrophy (Simmonds), inflammatory granular atrophy (Rössle), cytotoxic contracted suprarenal gland (Kovács), and so on.

The finding of intranuclear inclusion bodies in the cells of the adrenal cortex has stimulated the report of the following single case.

## REPORT OF CASE

*Clinical History:* J. N., Hospital No. A60474, a 53 year old Jewish female, was admitted to the New Haven Hospital on August 26, 1935, with a history of "unconsciousness" for several days. She died 20 hours after admission.

\* Received for publication November 22, 1935.



Her family and past histories, as far as could be elicited, were non-contributory. She was said to have been intelligent, very energetic, and had efficiently operated a real estate business. She received a blow in the left occipitoparietal region in April, 1935, when the car she was driving was in a collision. She continued to drive the machine and though it is reported that there were no immediate sequelae, she was observed at the Park City Hospital (Bridgeport) for several days without the disclosure of anything of significance. However, a train of symptoms appeared, consisting at first of complaints of weakness and "not feeling right." The patient's husband noticed that she was increasingly nervous and irritable, and she became unable to continue her work. No blood pressure data were obtained at this period. The condition progressed and by mid-July additional symptoms made their appearance — nausea, retching, vomiting and vague epigastric pain, apparently not related to food.

On August 7th she was admitted to the Bridgeport Hospital where she remained for 10 days. Physical examination, electrocardiography, gastro-intestinal X-ray series and the Graham gall-bladder test yielded nothing of significance. The hemoglobin was 83 per cent. Blood Wassermann and Kahn reactions were negative. The blood pressure is stated to have varied from 110/85 to 125/90 mm. Hg. on several occasions. The pulse was rapid (110-120 per minute), often thready. She was discharged with a diagnosis of traumatic neurosis.

Continued extreme restlessness, irritability and marked weakness necessitated a liberal use of sedatives (chloral hydrate, bromides and sodium amytal). Of the latter she received about 25 gr. from August 20th to August 22nd. She then gradually became semicomatose. Lumbar puncture revealed 20 cells per cmm. The Wassermann and Pandy tests and the colloidal gold curve were negative. On August 24th complete unconsciousness supervened, and although the neurological findings were vague, a diagnosis of probable subdural hemorrhage or brain tumor was made and the patient was transferred to the New Haven Hospital.

On admission the patient was completely unconscious. The temperature was 102.6° F., the pulse rate 140 per minute, the respirations 48 per minute, and the blood pressure 60/20. The upper teeth were absent. There was very slight edema of the left optic disc. The heart sounds were of poor quality. The lungs were clear and the abdomen soft. The neurological findings were indefinite: slight ptosis of the right eyelid, slight right facial weakness, faintly elicited abdominal reflexes, absent knee and ankle jerks and a positive right Oppenheim reflex.

Her course in the hospital was precipitously downhill. The pulse rate continued rapid at 160-180 per minute; the blood pressure dropped shortly after admission to 50/? and finally was not obtainable. The temperature rose to 104.5° F. Therapy consisted of large quantities of parenteral glucose and saline but death ensued 20 hours after admission. The patient, it is interesting to note, was anuric for 48 hours before death. There were two loose bowel movements, not further characterized.

The laboratory data showed a hemoglobin of 52 per cent with 3,900,000 red blood cells per cmm., 13,400 white blood cells per cmm., with a differential count of 58 per cent polymorphonuclear leukocytes and 35 per cent lymphocytes. The urine (2 cc. obtained by catheterization) was amber-colored, acid, negative for sugar and showed epithelial cells microscopically. The blood non-

protein nitrogen was 68 mg. per cent and the sugar 89 mg. per cent. Blood chlorides were 110 m.eq. (done after death, on a specimen of blood removed for other purposes, after considerable parenteral saline therapy). The blood Kahn reaction was negative.

Postmortem analysis of the liver showed 20 mg. of barbiturates to 500 gm. of liver. This was performed because amytal poisoning was suspected during the end of the clinical course.

#### POSTMORTEM EXAMINATION \*

*Gross Description:* The body was short, well developed and well nourished to the point of obesity. The skin was of a slightly dusky pallor. Although several skin areas, especially over the forearms, had a suggestive yellowish brown coloration, no definite punctate or diffuse pigmentation was seen either over the skin or mucous membranes. The scalp hair was abundant and somewhat coarse. The ears, eyes and nose were negative. The buccal mucosa was intact and was not evidently pigmented. The upper teeth were absent. Superficial examination of the chest, abdomen and perineum was negative. There was considerable subcutaneous adipose tissue, attaining a thickness of 5.5 cm. over the abdominal wall. The peritoneum was smooth and glistening and the viscera were all normally disposed. There was no evident enlargement of the mesenteric lymph nodes. The thymic region was entirely replaced by a large mass of adipose tissue. There were numerous, thin fibrous adhesions in the left upper pleural cavity.

The *heart* was small and weighed 255 gm. The musculature was a uniformly dark brown. The valves were thin and delicate. The coronary arteries were thin-walled and patent.

The *right lung* weighed 430 gm. and the *left* 375 gm.; both were covered with a glistening pink pleura. The tissue was uniformly soft and crepitant and the cut surfaces were moderately dry and spongy. Several patches of superficial atelectasis were found over the posterior aspect of both lower lobes. The bronchi and the pulmonary vessels showed nothing of note.

The *spleen* weighed 125 gm. and was moderately firm. The cut surface was slightly moist, a homogeneous crimson, and liberally peppered with translucent gray malpighian corpuscles. The capsule was delicate.

The *stomach* and *duodenum* showed nothing of note.

\* Autopsy No. 3517.

The *gall-bladder* was thin-walled and contained 50 cc. of semiviscid green-brown bile.

The *pancreas* weighed 90 gm. and was normal.

The *liver* was normally shaped and weighed 1290 gm. The capsule of Glisson was uniformly glistening, thin and transparent and through it a bright, slightly brownish yellow parenchyma was visible. On section the areas around the hepatic veins were dark red and stood out brilliantly against the diffuse yellow background, thereby accentuating the usual hepatic lobulation.

The *right kidney* weighed 155 gm. and the *left kidney* 165 gm. Both were firm with thin capsules which stripped easily, exposing a brownish to reddish orange-tinted, smooth parenchyma. The cortex averaged 7 mm. in width and was distinctly demarcated from the medulla. The striations were regular but showed some blurring. The pelves were small. No abnormality was detected in the renal vessels. No urine was found in the small bladder. Patent ureteral orifices opened into thin and delicate ureters.

The *uterus* was small and not unusual. The cervix revealed a few scattered, small Nabothian cysts. The *vagina* was not remarkable.

Each *ovary* measured 2 by 1 by 1 cm., was ovoid and firm; the surface was corrugated, glistening and yellow-white. The *Fallopian tubes* were normal.

The *tongue* showed little except smooth and shiny lateral surfaces in its anterior portion.

Nothing unusual was seen in the *esophagus*, *larynx* or *trachea*.

The *intestinal wall* was thin throughout with an intact serosa. The mucosa was velvety. Scattered, fresh petechial hemorrhages were noted in the mucosa of the small intestine, and a few confluent superficial areas in that of the large intestine.

The *aorta* and its major divisions were elastic. A few intimal patches of soft atheroma were present.

Careful examination failed to reveal any evidence of old or recent trauma in the *scalp* or any trace of recent or old fracture in the *calvarium* or base of the skull. The *brain* presented no abnormality externally or in multiple sections. The tentorium was slightly calcified. The *paranasal sinuses* were dry and pink.

The *thyroid* was of the usual butterfly shape and weighed 25 gm. Externally and on section, spongy reddish brown areas alternated with firm yellowish gray areas. There was no grossly visible colloid.

The *parathyroids* were not recovered, even after careful search.

Each *adrenal* was firmly embedded in considerable adipose tissue. Both glands were evidently much reduced in size — the right weighed 4 gm. and the left 2 gm. The right was flattened and measured no more than several millimeters in its widest cross-section. The left was compact, elongated and triangular in cross-section. The glands were firm and externally a mottled gray-brown. Multiple cross-sections failed to reveal any traces of the usual architecture. The medulla was alternately dark brown and translucent gray. In some sections the medulla was contiguous with the capsule, in others irregular, small, yellow-gray and pale brown islands or vaguely interlacing strands formed what were apparently cortical remnants. None of the usual yellow color was seen in any of the cross-sections.

The *hypophysis* was slightly cup-shaped but revealed no gross abnormality.

#### MICROSCOPIC EXAMINATION

Tissues were Zenker-fixed and stained with hematoxylin-eosin, unless otherwise noted.

The *heart* appeared normal except for a slight increase in the quantity of perinuclear lipochrome granules.

The *lungs* were not remarkable.

The *spleen* showed a very cellular pulp. The majority of the cells were mononuclear — macrophages, plasma cells and lymphocytes — but numerous polymorphonuclear leukocytes, focally concentrated, and a not inconsiderable number of eosinophiles were present. Scattered granules of intra- and extra-cellular golden yellow pigment were seen. The malpighian corpuscles were numerous with large germinal follicles, the central portions of which were necrotic.

In the *pancreas* the only abnormality was one small focal area of fibroblastic proliferation and lymphocytic infiltration.

The *liver* showed extensive fatty deposition in all the cells, especially marked periportally. The architecture was otherwise not disturbed.

The *kidneys* showed a well preserved architecture. The epithelial cells in the proximal convoluted tubules were swollen and contained numerous small fat droplets, as demonstrated with Sudan III.

A preparation through the *colon* showed fresh hemorrhages in the

mucosa and many macrophages containing golden yellow pigment in the tunica propria.

The *diaphragm* was normal histologically.

A section through the anterior portion of the *tongue* revealed a moderate degree of fibrosis.

Preparations through both *ovaries* showed the usual atrophic ovarian tissue.

The *thyroid* consisted largely of small follicles, frequently with almost absent lumens, either devoid of colloid or containing a material that stained green in the Masson trichrome preparation, in contradistinction to red of normal colloid. The epithelium was largely cuboidal, occasionally columnar and well preserved. None of the infoldings and papillary projections usually associated with hyperplasia was seen. The connective tissue stroma appeared thickened in only a few areas, but focal collections of lymphocytes were frequent. Many of these focal lymphocytic infiltrations showed central lymph follicle formation (Fig. 1).

The *hypophysis* was sectioned serially and presented no abnormalities.

Numerous histological preparations of the *brain* failed to reveal any abnormality.

Many complete cross-sections, including both *adrenals*, were studied and only presented minor variations in the microscopic picture. The capsule was intact and not thickened. The general architecture was strikingly disorganized and this was almost entirely due to the cortical damage. Instead of the usual rows of parallel cell cords the cortex was frequently absent, so that the medulla was adjacent to the capsule on either side (Fig. 2). In other areas adenomatous-like areas of cortical cells vaguely arranged in irregular cords or masses were seen (Fig. 3). Or frequently, isolated cells or small groups of cells were embedded in a fibrous connective tissue background. The latter did not appear to be increased and this view was substantiated in the preparations stained with Mallory's anilin blue collagen stain. This connective tissue was rich in capillaries and was most abundant in the regions where the cortex was missing. The entire tissue was infiltrated with lymphocytes and plasma cells but not uniformly, some areas being densely infiltrated while others contained scattered individual cells. The medulla was everywhere abundant and consisted of elongated cells with a highly vascularized

connective tissue stroma. Chromaffin granules were rare. Small focal areas of lymphocytic infiltration were present in scattered areas. There was no perceptible abnormality of the larger or smaller blood vessels.

The individual cortical cells showed striking variations in size and shape. Many of the cells were of the usual size and somewhat polygonal but the majority were large, many enormous, with rounded or irregular contours. Even with the ordinary high power magnification, the cytoplasm presented variable appearances: it frequently stained a homogeneous translucent pink, sometimes pale, sometimes intense; in other areas it was finely or coarsely granular; and in still others was delicately vacuolated and reticular. The cytoplasmic borders were frequently broken and the frayed, granular cytoplasmic edges projected in irregular streamers. Many cells contained fine yellow-brown granules, resembling chromaffin granules, evenly distributed throughout the cytoplasm. A Sudan III stain showed that most of the cortical cells were devoid of fat, but haphazard groups contained moderate amounts of finely divided intracytoplasmic globules.

The nuclei of these cells were roughly round in contour but showed extensive variations in size and structure. Not infrequently, two or three nuclei were seen in a single cell. Very few of these appeared normal, *i.e.* with evenly distributed, dark blue staining chromatin granules, a few scattered acidophilic or amphophilic granules, and a slightly eccentric or central dark blue staining nucleolus. The majority of nuclei presented changes which, despite an apparent diversity, definitely suggested various stages in a process. It is obviously precarious to describe a process in terms of end-results, but an attempt will be made to describe the findings in the most plausible sequence. The most common — and this was seen in varying degree in most of the nuclei — was a partial disruption of the dark blue staining chromatin network, with a tendency for the individual chromatin granules to migrate peripherally toward the nuclear membrane. The one or two nucleoli usually present participated in this migration. In not a few of these nuclei the amount of chromatin seemed to be appreciably decreased. Scattered throughout the nuclear background, diffusely but with some focal concentration, were pale pink staining granules, discrete or partially and irregularly fused. The greatest concentration of this acidophilic material was,

as a rule, toward the center. The nuclear membrane was often slightly thickened. Not infrequently nuclei showing these changes were astonishingly large with a relatively thin nuclear membrane. Such nuclei were generally found in correspondingly large cells. There was no precise correlation between nuclear and cytoplasmic change but nuclei showing the above changes in a high degree were usually surrounded by very granular, pale staining cytoplasm with frayed edges. Next in frequency were nuclei in which the peripheral chromatin migration was more pronounced, although even here granules of chromatin were occasionally still present in the central area. The acidophilic material in these nuclei stained a somewhat deeper pink and the individual granules had largely fused into an amorphous mass. Occasionally one or two small, round, compact acidophilic inclusions were surrounded by loose amorphous granules. In this type of nucleus there was frequently a suggestive halo between the central pink staining material and the peripherally placed chromatin. As a rule, these nuclei were found in groups. The least frequent type of nucleus with acidophilic inclusions was found only after careful search. Roughly speaking, one, two or three were seen in each section. These were of two types: the first (Fig. 4) in which the nuclear membrane was thickened or wrinkled, the chromatin largely peripherally located with considerable amounts of it seemingly fused to the inner wall of the nuclear membrane, a clear halo just within the chromatin layer and a round, deep, pink staining, slightly mottled inclusion, occasionally containing a blue staining area, occupying the central part of the nucleus; and the second (Fig. 5) in which the central inclusion was surrounded by a thin, dark blue staining, apparent membrane, on which chromatin granules were margined. In the latter type of cell a halo was absent.

No noteworthy differential characteristics were brought out by special stains. The inclusions were stained bright red by Mallory's anilin blue collagen stain, reddish brown by Masson's trichrome stain (ponceau-acid fuchsin-lichtgrün), and pale pink by eosin-methylene blue and by the Giemsa stain.

Nuclear inclusions were carefully looked for but to no avail in all of the sections of the other tissues in this case and in many adrenals from cases of varied diseases.

## DISCUSSION

The clinical history in this case presents several features worthy at least of brief comment. Detailed analyses are available in a few of the extended reports in the literature.<sup>1,4</sup> The apparently sudden onset of a disease certainly not *prima facie* functional, following trauma with no evident physical consequences, seems difficult to understand. However, that terrific emotional strains or minor accidents will frequently precipitate an Addison's disease associated with "atrophy," as well as with tuberculosis of the adrenals, is evident to everyone after a review of the literature. Marañón<sup>8</sup> has recorded numerous instances. Patients with Addison's disease, it is well known, are extremely poor surgical risks. Can it be that the disease process has progressed subclinically until a sudden demand on the adrenals demonstrates dramatically that they have lost their margin of safety? Swingle and Parkins<sup>9</sup> have recently made an experimental approach to the problem. By subjecting normal and adrenalectomized, healthy vigorous dogs to various degrees and forms of trauma (surgical, testis, intestinal and muscle), they demonstrated an extreme susceptibility to even slight trauma in the adrenalectomized animals. Such animals develop profound and fatal shock rapidly. Cortin, before or after the trauma, will restore the animal. Apparently, the only variable factor is cortin, of which the adrenalectomized animal has no reserve.

The fact that the blood pressure of the subject of this report was apparently normal until the terminal crisis is not remarkable and has been noted frequently enough. The value of this as a clinical differential point between "atrophy" and tuberculosis<sup>10</sup> has been denied.<sup>1</sup>

The relatively rapid progress of the illness observed in this patient is interesting. Though an acute course of several months or less is not usual, it occurs with both tuberculosis and "atrophy" but is much less frequent in the latter.<sup>1</sup>

Pigmentation, it is well known, is not a *sine qua non* of Addison's disease. Guttman furthermore has collated the material from the literature showing that the course is significantly long where pigmentation is marked, and is generally of brief duration where weakness is the predominant symptom.



The pathological features of this case, except for the nuclear inclusion bodies, fit completely the numerous descriptions given by various authors. The adrenals are small and shrunken, and externally in a miniature way frequently resemble cirrhosis of the liver. There is complete distortion of the usual architecture on cut section. Microscopically the lesions are limited almost exclusively to the cortex, the medulla generally showing, if any lesion at all, a variable amount of lymphocytic infiltration. The cortex may be completely absent or replaced by nodular hyperplastic islands of cells, or isolated cells embedded in an infiltration of lymphocytes or plasma cells. The cells vary in size: the cytoplasm is generally poor in lipid content and frequently has irregular borders. The nuclei show marked variability in size, shape and chromatin content. The fibrosis is slight and is evidently a contraction phenomenon.

The thyroid is almost constantly reported to show lymphocytic infiltration with the formation of germinal centers, sometimes so extensive that it becomes difficult to identify the tissue as thyroid. The exact significance of this is not known; it appears to be a secondary phenomenon, although many and frequent debates occur in the German literature concerning the relative importance of the adrenal and thyroid.<sup>11, 12, 13, 14</sup> The changes in the other endocrine glands are inconstant. Although vacuolar degeneration, disappearance of the cytoplasmic granules and diminution in the number of the basophile cells of the pituitary are reported,<sup>12</sup> serial sections showed a normal histology.

Although no history of the classical symptoms of diarrhea was obtained in the present case, the fresh petechial hemorrhages, in addition to the numerous macrophages heavily laden with old blood pigment seen in the tunica propria of the intestinal mucosa, offer presumptive evidence of intestinal involvement.

The wearisome polemics in the literature concerning the relative importance of cortex and medulla in the pathogenesis of Addison's disease need interest us no longer. It has become increasingly clear from reports such as the present one that, even though additional symptomatology may be associated with medullary changes, typical Addison's disease can be produced by the destruction of the adrenal cortex alone.

The numerous discussions of the etiology of adrenal "atrophy" are distinguished by a diversity of opinion as bewildering as it is uncon-

vincing. Syphilis, tuberculosis, congenital hypoplasia, "cytotoxins" circulating in the blood, chronic inflammation — all have been analyzed with thorough inconclusiveness. The present case, although isolated, offers at least suggestive evidence of a definite etiological factor. The selective involvement of specific cells associated with a disease process, the slow necrosis, hyperplasia and hypertrophy of these adrenal cortical cells, the apparently secondary inflammatory reaction and the relatively insignificant degree of fibrosis — all of these characteristics in addition to the finding of intranuclear inclusions offer presumptive evidence, at least, for another interpretation.

### SUMMARY AND CONCLUSIONS

1. A case of "atrophy" of the adrenal glands in a 53 year old woman is reported. The apparently sudden onset after a slight trauma and the rapid clinical course are noteworthy.

2. Intranuclear inclusion bodies were found in the cortical cells of the adrenal glands. Together with other characteristics noted, these may offer possibly another etiological interpretation.

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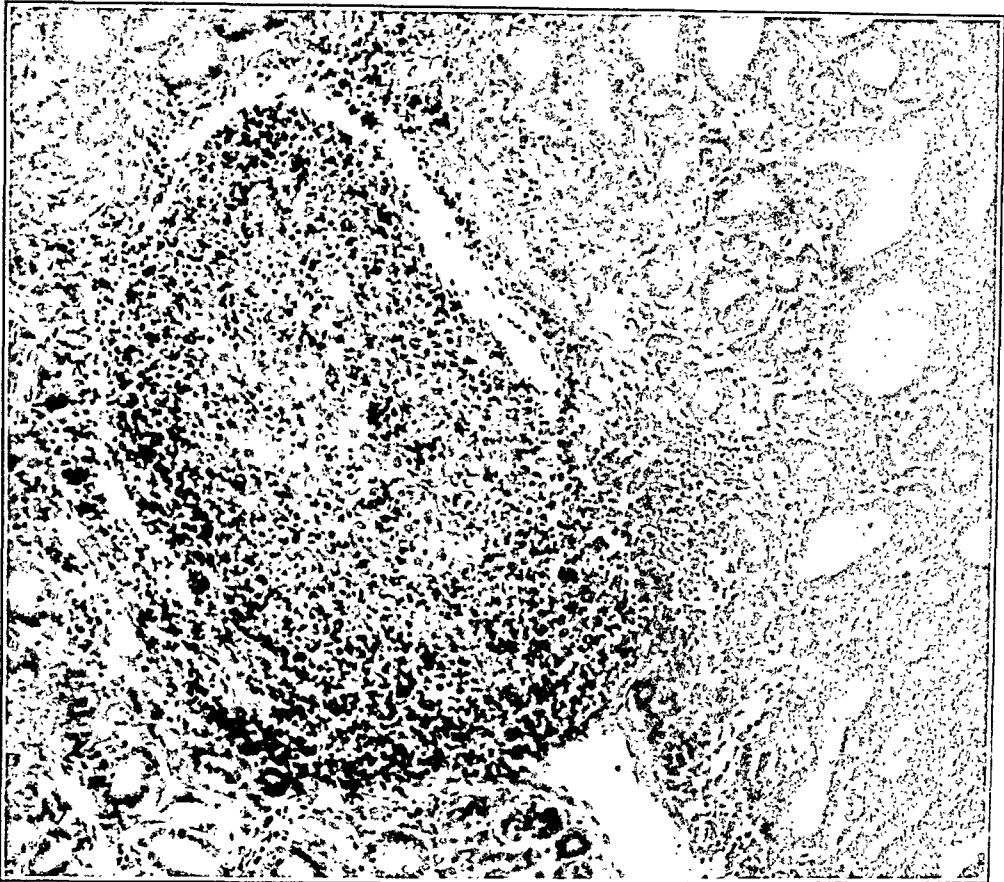
## DESCRIPTION OF PLATES

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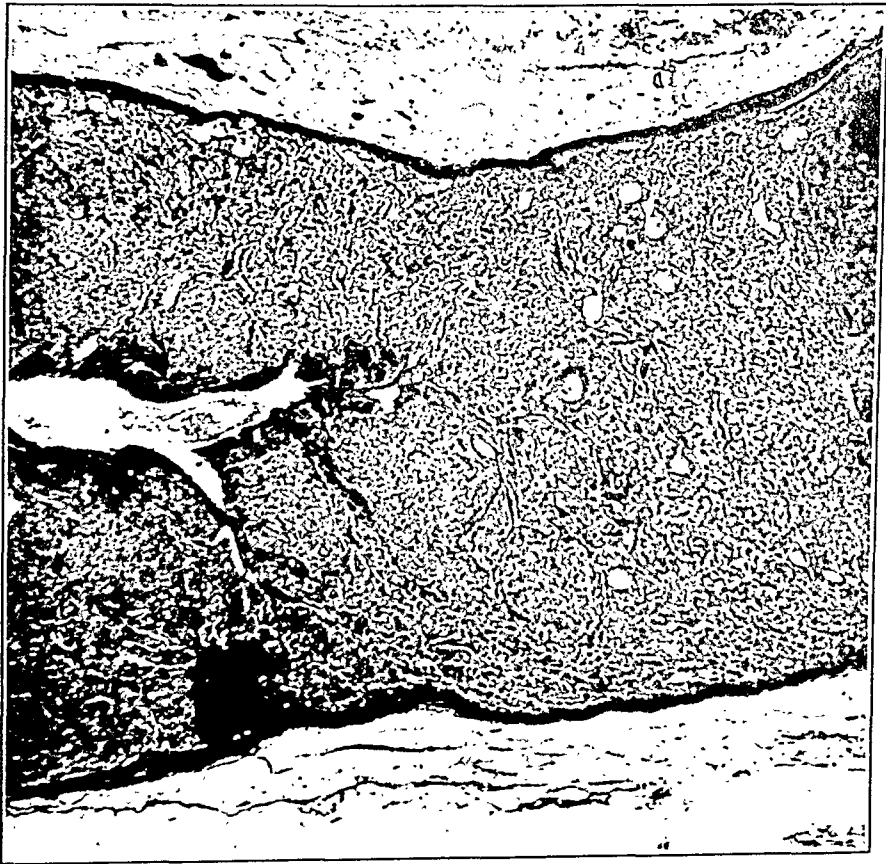
### PLATE 48

FIG. 1. Thyroid gland showing lymph follicle formation.  $\times 125$ .

FIG. 2. Section of right adrenal showing complete absence of cortex.  $\times 26$ .



I



2



# ADENO-ACANTHOMA SARCOMATODES OF THE MAMMARY GLAND \*

## REPORT OF A CASE, WITH A CRITICAL REVIEW OF THE LITERATURE ON SQUAMOUS EPITHELIUM IN INTRAMAMMARY TUMORS

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Tumors of the mammary gland have been so thoroughly studied, classified and recorded as to leave little excuse for further pathological reports. However, after a critical review of the literature on histomorphologically malignant mixed tissue tumors of the breast it is believed that the case reported here is almost unique.

Primary intramammary epidermoid carcinoma in which the epithelium had no demonstrable connection with the skin of the breast has been reported by Troell <sup>1</sup> (2 cases), and Orth,<sup>2</sup> Ribbert <sup>3</sup> and Delbet and Mendaro,<sup>4</sup> 1 case each.

Malignant degeneration of intramammary cholesteatomas was observed by Kaufmann,<sup>5</sup> Lecène,<sup>6</sup> Konjetzny <sup>7</sup> and Lahm<sup>8</sup> (1 case each).

True intramammary adeno-acanthoma is very rare, only the single cases of Orth,<sup>2</sup> Brocq, Wolf and Giet,<sup>9</sup> and of Loeb,<sup>10</sup> being on record.

Kreibig <sup>11</sup> has reported 1 case that had a composite structure of adenocarcinoma, sarcoma and acanthoma.

The case reported below presented such a composite structure with a preponderance of the acanthomatous element.

### REPORT OF CASE

*Clinical History:* The patient, J. H. W., a white, unmarried, female nurse, 60 years of age, was first admitted to the Marine Hospital, Seattle, Washington, on April 29, 1935, complaining of a tumor in the right breast.

The past history revealed no serious illnesses since childhood. The patient had a normal menopause at the age of 46. In 1918, while nursing in France, she noted a small lump in the right breast, in approximately the same area as

\* Received for publication November 26, 1935.

the present one, which she says was present about a year. She forgot about this and did not remember the incident until one of her friends, a nurse who had seen the mass, reminded her of this fact during her present hospital admission.

She had been struck in the breast in January, 1935, and the following day noticed a small tumor in the upper outer quadrant of the right breast. The mass was about 3 by 3 cm. when she first noticed it, and the skin over it had a bluish color. It rapidly increased in size. On April 29, 1935, about an ounce of turbid, slightly blood-tinged fluid was aspirated from the mass and a biopsy was taken.

Physical examination disclosed a fairly well developed and nourished white female of 60 years, in good general condition. Other than false upper teeth and poor lower teeth showing considerable dental caries and pyorrhea alveolaris, the examination was negative except for the local condition in the breast.

In the upper outer quadrant of the right breast was a rounded firm mass 7 cm. in diameter, which was tightly adherent to the skin and to the breast tissue immediately under it, but apparently movable over the pectoral muscles. The skin covering the mass was red over an area 10 cm. in diameter. The remainder of the breast and the nipple appeared normal. There were no palpable axillary or supraclavicular nodes and there was no evidence of distant metastases.

Following the report of the biopsy, a Halsted-Meyer type of radical mastectomy was done. The entire breast, both pectoral muscles, axillary contents and a wide area of skin surrounding the tumor in the upper outer quadrant of the breast were removed. It was possible to approximate the skin edges.

The patient made an uneventful postoperative recovery. The major portion of the wound healed by primary union. Two months postoperatively she had gained 12 pounds in weight, appeared perfectly well and had no evidence of local or regional recurrences and no evidence of distant metastases.

*Biopsy Specimen:* Infiltrating angular masses and branching strands of flat squamous epithelial cells were irregularly disposed in a diffuse fibrosing and fibroblastic stroma. Some islets showed intercellular prickles, some showed variable degrees of keratinization and occasionally cornified concentric cell nests were seen. No other elements were present.

*Mastectomy Specimen:* The formalin-fixed specimen represented practically the whole mammary gland. The breast, laid open by an incision over the tumor, disclosed a roughly quadrangular, solid, hard grayish mass, 3 by 4 cm., which appeared to abut on the skin in several places and tapered off into the adipose tissue elsewhere. The surface was studded with many pin-point to pin-head sized, concentrically laminated, mother-of-pearl granules. In the base of the tumor a smooth-walled cyst 1 cm. in diameter was present. Embedded in the fat of the breast in the vicinity of the tumor were seen several smaller cysts.

## HISTOLOGICAL EXAMINATION

Sections of the whole tumor were prepared from three different areas, including the overlying skin and adjacent breast tissue. Nine sections were prepared from other portions of tumor, breast tissue and pectoral muscles. They were stained with Weigert's iron chloride hematoxylin and Van Gieson's picro-fuchsin, Mayer's acid hemalum and eosin, a modification of the Romanowsky stain for the demonstration of intercellular bridges, Gram's stain for keratohyaline granules and Weigert's resorcin-fuchsin for elastic fibrils.

The skin of the breast was normal and separated from the tumor proper by a zone of dense acellular connective tissue 9 to 30 mm. in thickness. The tumor was not encapsulated but tapered off into the fibro-adipose tissue of the breast.

The structure of the tumor varied, but it was predominantly an epidermoid carcinoma. All stages of keratinization were present, grading from diffusely infiltrating branching strands and cords of embryonal epithelial cells to completely cornified, concentric squamous cell masses. Small solitary and large confluent acanthomatous cysts filled with cornified pearls occurred throughout the specimen. These cysts were lined by keratinizing squamous epithelium, from the basal layers of which finger-like projections of variable size and thickness extended into the stroma. Cystic ducts and deep seated cysts in areas of the tumor were lined partly by cylindrical or cubical epithelium and partly by squamous epithelium or bulky cornifying masses that projected into the lumens. In these areas gradations of the cylindrical or cubical epithelium to squamous epithelium were occasionally encountered. Here the stroma was more or less sclerosed and infiltrated by disorderly neoplastic acini lined by hyperchromatic cubical epithelium. Here and there were small cysts more or less filled with papillary and tubular masses. In one area the wall of a cyst was perforated and the glandular growth infiltrated the adjacent tissues.

The character of the stroma also varied. In the adenocarcinomatous portion of the tumor it consisted of ordinary fibrocellular or acellular sclerosing connective tissue. In the anaplastic epidermoid areas highly cellular fibroblastic connective tissue prevailed. Here the fibroblasts exhibited large hyperchromatic nuclei, occasionally multiple nuclei and less often mitosis. In some areas strands of



embryonal or anaplastic squamous tumor frayed out and often blended with the sarcomatous stroma, requiring careful study for differentiation.

In areas in which the acanthomatous cysts had broken down and liberated degenerated cornified material the stroma was obviously of the granulation tissue type and was infiltrated by plasma cells and lymphocytes. Rarely it showed foreign body giant cell granulomas which occasionally contained cholesterol crystals.

An axillary lymph node showed only sinus endothelial hyperplasia.

## DISCUSSION

Occasionally, variable stretches of the cubical or columnar epithelium lining the ducts, clefts or cysts, are replaced by squamous epithelium in the large cystadenomatous tumors of the breast. This may show all of the characteristics of cutaneous epithelium.

The majority of recorded observations of squamous epithelium in intramammary tumors have been reported as "cholesteatoma." Müller<sup>12</sup> was the first to describe cysto-sarcoma-phyllodes, and also the first to point out that these tumors might contain onion-like, concentric, epithelial pearly formations which he designated as "cholesteatoma."

There is much latitude in the employment of this term in the literature. In the present connection only those structures are included that are composed of, or contain, more or less concentrically laminated or degenerated, keratinized epithelial squames enclosed in a wall of stratified squamous epithelium.

Among the 31 recorded observations 21 were reported associated with cysto-sarcoma-phyllodes. In the majority the cholesteatomas were macroscopically recognizable. They were generally solitary but sometimes occurred in large numbers. They were embedded in the connective tissue of the tumor as globular, pin-head to cherry sized formations, having a silvery or mother-of-pearl appearance. On section they often presented a laminated, onion-like configuration.

Microscopically the lining epithelium showed variable degrees of stratification, keratinization and desquamation. In the cases of Konjetzny,<sup>7</sup> and Haardt,<sup>13</sup> papillae were formed. In 17 of the reported cases the epithelium exhibited intercellular prickles, kerato-

hyaline granules and cornification. Stoerk and Erdheim,<sup>14</sup> and Konjetzny,<sup>7</sup> found elastic fibrils in the stroma adjoining the cholesteatoma. Skin adnexa have not been observed.

These epidermoid formations were first described by Cooper,<sup>15</sup> Müller,<sup>12</sup> and Virchow.<sup>16</sup> Subsequent reports may be found by Model,<sup>17</sup> Bauchet,<sup>18</sup> Klob,<sup>19</sup> Schmidt,<sup>20</sup> Kürsteiner,<sup>21</sup> Haeckel,<sup>22</sup> Grohé,<sup>23</sup> Wilms,<sup>24</sup> Stumpf,<sup>25</sup> Beneke,<sup>26</sup> Stoerk and Erdheim,<sup>14</sup> Coyne and Brandeis,<sup>27</sup> Kuru,<sup>28</sup> Walther,<sup>29</sup> DeBoucaud and Nadal,<sup>30</sup> Prym,<sup>31</sup> Konjetzny,<sup>7</sup> Gorham,<sup>32</sup> Lahm,<sup>8</sup> Bouchet and Martin,<sup>33</sup> Haardt,<sup>13</sup> Turco,<sup>34</sup> Biebl,<sup>35</sup> Kückens,<sup>36</sup> Oliver and Major,<sup>37</sup> Kaufmann,<sup>5</sup> Ribbert,<sup>3</sup> Deaver and McFarland,<sup>38</sup> Ewing,<sup>39</sup> and Schultz-Brauns.<sup>40</sup>

Malignant degeneration of cholesteatomas has been reported by Kaufmann,<sup>5</sup> Konjetzny,<sup>7</sup> and Lahm.<sup>8</sup> The case of Lecène<sup>6</sup> probably belongs in this category also.

Kaufmann<sup>5</sup> observed an epidermoid cyst as large as a hen's egg in the breast of a woman, aged 45 years. It was deep in the breast and had degenerated to form an epidermoid carcinoma. There was also present a coincident carcinoma simplex.

Konjetzny<sup>7</sup> described the case of a woman, aged 34 years, who discovered a movable tumor in her right breast during a pregnancy. The amputated breast disclosed a tumor 15 by 8 cm., which contained a fist sized cholesteatoma. The epidermoid wall of the cyst suffered malignant degeneration and gave rise to typical prickle cell and basal cell carcinoma, respectively. There was no demonstrable connection of the epidermoid elements with the skin of the breast.

Lahm<sup>8</sup> reported a cholesteatoma carcinomatosum in a nullipara, aged 34 years, who had noticed a growing nodule in the right breast for 1½ years. It was encapsulated and easily enucleated. Cut section disclosed a large cyst filled with atheromatous mush, and multiple small cholesteatomas. Microscopically it was an intracanalicular fibro-adenoma containing multiple cholesteatomas lined by typical epidermoid epithelium. In areas the cylindrical epithelium of the ducts was replaced by squamous epithelium. Proliferations of squamous epithelium infiltrated the stroma of the tumor in many areas. The skin of the breast was free and intact.

Lecène's<sup>6</sup> case probably represents a cholesteatoma that had progressed to advanced necrosis before malignant proliferation began. The patient, a woman, aged 54 years, developed a hen's egg sized tumor in the center of her breast in the course of 2 months.

The skin was intact and the nipple normal. The tumor presented a soft central mass and was enclosed in a tough capsule. Microscopically the skin was normal and separated from the tumor by subcutaneous adipose tissue and the connective tissue capsule. The central necrotic mass was margined by a "genuine dermal papilla" covered by typical epidermis. Masses of malpighian epithelium were disseminated throughout the stroma and some of them showed typical epithelial tumor giant cells. The stroma of the tumor presented a sarcomatous appearance of the epulis type.

In another group of cases isolated foci of presumably benign squamous epithelium are reported in tumors of the breast.

Stumpf's<sup>25</sup> case, a cysto-sarcoma-phyllodes, contained islets of squamous epithelium scattered in the stroma in the vicinity of ducts and cysts lined partly or completely by squamous epithelium. Stoerk and Erdheim<sup>14</sup> described scanty islets of squamous epithelium in a fibro-adenoma. In Herrenschildt's<sup>41</sup> case the tumor was separated from the skin of the breast by a distinct band of connective tissue. Its margin was formed of typical epidermis and its interior contained non-keratinizing squamous masses. Ewing<sup>39</sup> studied a case in which the glandular and squamous epithelium were about equally divided and transformation of one type into the other seemed obvious. The case of Johnson and Lawrence<sup>42</sup> seems comparable with Ewing's. D'Allaines and Hiely<sup>43</sup> reported a benign encapsulated fibro-adenoma in which strands of keratinizing prickle cell epithelium were disposed throughout the stroma. Pearl bodies were formed. Small collections of atypical squamous cells were also seen. The authors regarded this as a benign tumor because it was encapsulated.

Pure intramammary squamous carcinoma having no demonstrable connection with the skin of the breast is rare.

Between 1898 and 1907 Troell<sup>1</sup> examined 185 cancers of the breast, among which there were 2 cases of squamous carcinoma. His first case, a woman, aged 57 years, had a cystic tumor one-half the size of a fist, situated beneath the skin. Microscopically the tumor was a typical acanthoma. The skin was normal and in no way related to the tumor. The second case showed a fist sized, sharply circumscribed tumor, which histologically was a squamous carcinoma having no demonstrable relation to the skin or its appendages. Both cases represented deep seated primary growths.

Orth<sup>2</sup> casually called attention to a primary acanthoma of the mammary gland which was later reported in detail by Calderara.<sup>44</sup> A mammary gland the size of a fist contained in its depth a grayish white, hard streaked mass 10 by 3 cm. It was embedded in a thick layer of adipose tissue and did not infiltrate the underlying muscle. Microscopically the tumor was confined to the glandular portion of the breast. It presented a lobular structure of anastomosing solid strands and branches of squamous epithelium which infiltrated the connective tissue. The epithelium showed various grades of keratinization and pearl body formation. No metaplastic relationship with the glands could be demonstrated and there was no evidence of any connection with the milk ducts or the skin.

Ribbert<sup>3</sup> described a tumor the size of an apple which infiltrated the tissue of the breast and showed hard and soft areas. The hard areas showed the structure of an adenoma infiltrated by epidermoid carcinoma. In one area cholesteatomatous cysts were present. The soft areas were decidedly polymorphous cell sarcoma which infiltrated the adenomatous portion of the tumor as well as the breast tissue.

Busacchi and Miani<sup>45</sup> described 2 cases of pavement cell carcinoma of the breast. The histological features, both from the descriptions and the accompanying photograph, are not convincing.

Many duct cancers of the breast, notably comedo carcinoma, present an alveolar structure, ducts distended with necrotic debris, a lining of large, flattened, atypical hyperchromatic cells showing more or less stratification and mutual deformation, resulting in a picture resembling pavement epithelium. These peculiar mechanical relations are but rarely mentioned in the literature, but that they not infrequently result in epithelial types which occasionally defy classification is the experience of every pathologist.

Delbet and Mendaro<sup>4</sup> very carefully studied the case of a woman, aged 62 years, who had a rapidly growing tumor that involved most of the left breast. The skin was intact; it showed no orange peel effect. The breast was amputated. Microscopically the tumor was a typical keratinizing prickle cell epithelioma. There were no grounds for assuming that the tumor was of cutaneous origin or metastatic. Ten years later the patient was alive and in good health.

The cases of Nadal<sup>46</sup> and of Costantini<sup>47</sup> probably belong in the same category as to their source of origin — namely from the larger milk ducts.

The skin was intact and the nipple normal. The tumor presented a soft central mass and was enclosed in a tough capsule. Microscopically the skin was normal and separated from the tumor by subcutaneous adipose tissue and the connective tissue capsule. The central necrotic mass was margined by a "genuine dermal papilla" covered by typical epidermis. Masses of malpighian epithelium were disseminated throughout the stroma and some of them showed typical epithelial tumor giant cells. The stroma of the tumor presented a sarcomatous appearance of the epulis type.

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The cases of Nadal<sup>46</sup> and of Costantini<sup>47</sup> probably belong in the same category as to their source of origin — namely from the larger milk ducts.

Nadal's patient, a woman aged 80 years, presented a very large tumor that had invaded the entire mammary gland. In areas the tumor was an ordinary scirrhous carcinoma. In its greater part it was made up of thick plexiform epithelial cords in the midst of which extremely diffuse and irregular epidermoid involution was seen. Pearl bodies were rare.

Costantini <sup>47</sup> reported the contemporary bilateral development of two distinct carcinomatous tumors in a woman aged 51 years. In 2 months the tumor of the left breast grew to the size of a man's fist. Microscopically the broad cords and branches of thickened epithelial cells involved extensive areas of the breast. Every gradation from neoplastic cylindrical epithelium to prickle cell epithelium containing keratohyaline granules and undergoing keratinization and pearl body formation was demonstrable. The tumor appeared to develop from the epithelium of the milk ducts.

Tumors composed of mixed glandular and epidermoid carcinoma are often designated as adeno-acanthoma.

Orth <sup>2</sup> briefly called attention to another case which was reported in detail by Calderara.<sup>44</sup> The specimen, a breast the size of a child's fist, showed a normal skin covering separated from the underlying tumor by a thick layer of adipose tissue. The tumor was about the size of a fifty-cent piece, 2 cm. thick, hard and grayish white. Microscopically it was an adenocarcinoma inseparably mixed with an acanthoma, with obvious transitions from cylindrical to squamous epithelium. The skin and underlying adipose tissue were normal.

The case of Brocq, Wolf and Giet <sup>9</sup> presented the clinical feature of subacute mastitis or carcinomatous mastitis. The patient, a woman aged 45 years, had a fluctuating temperature and an acutely inflamed right breast which excreted pus from the nipple. A firm movable mass raised the suspicion of a neoplasm, so the breast was amputated. The tumor was a mucus adenocarcinoma grading into squamous carcinoma. The axillary glands showed atypical non-colloid tumor.

Loeb <sup>10</sup> reported the case of a woman who developed a fist sized hard tumor in her right breast and had large glands in the same axilla. A biopsy disclosed an adenocarcinoma, whereupon a complete operation was done. The primary tumor was predominantly an adenocarcinoma showing all stages of transition to epidermoid carcinoma. The axillary nodes also showed adeno-acanthoma.

Kreibig's<sup>11</sup> case is somewhat similar to ours. A large cystic tumor was removed from the left breast of a woman aged 52 years. In the wall of the largest cyst a protruding nodular tumor was present. Prof. Stoerk examined the specimen histologically, and made a diagnosis of tubular carcinoma arising in an intracystic papilloma and containing isolated islets of squamous epithelium. Fifteen months later the patient returned with a recurrence the size of a man's fist. A complete operation was done. The histological examination disclosed a polymorphous cell sarcomatous stroma and areas of adenocarcinoma showing complex intracystic papillary formations. The cysts and spaces were lined partly by cylindrical epithelium and partly by large stretches of cornified squamous epithelium. Scattered throughout the stroma there were large and small, solid, concentrically stratified keratinizing masses of squamous epithelium. The axillary lymph nodes showed only adenocarcinoma.

It thus appears that the sarcoma developed at a later date and that the epidermoid elements proliferated along with the adenocarcinoma.

Sarcoma may arise in the matrix of any tumor and a variety are reported in the mammary gland. Unfortunately, many of these are errors, authors having included rather cellular adenofibromas and cysto-sarcoma-phyllodes, which have no distinct malignant qualities but have reached a large size because of their long duration (Ewing,<sup>39</sup> Lee and Pack,<sup>48</sup> Kaufmann,<sup>5</sup> and Schultz-Brauns<sup>40</sup>). However, the cellular pseudosarcomatous areas occasionally become really sarcomatous (Prym,<sup>31</sup> Gorham,<sup>32</sup> Haardt,<sup>13</sup> Bouchet and Martin,<sup>33</sup> Ribbert,<sup>3</sup> Deaver and McFarland,<sup>38</sup> Ewing,<sup>39</sup> and Schultz-Brauns<sup>40</sup>). Kreibig<sup>11</sup> and Deaver and McFarland<sup>38</sup> expressed the opinion that the great majority of mammary sarcomas are complicating. They may make their appearance months or years after the tumors have existed (Deaver and McFarland,<sup>38</sup> Kreibig,<sup>11</sup> and Schultz-Brauns<sup>40</sup>). In the case reported here it is believed that the sarcomatous process was secondary to the acanthoma.

#### COMMENT

The origin of squamous epithelium in intramammary tumors is of considerable pathological interest. The embryogenesis of the gland permits the very reasonable deduction that in its first anlage an



ectodermal bud is snared off and later develops. Among others, Schmidt,<sup>20</sup> Wilms,<sup>24</sup> Ribbert,<sup>3</sup> Orth,<sup>2</sup> Stoerk and Erdheim<sup>14</sup> and Konjetzny<sup>7</sup> subscribe to Conheim's doctrine. This conception seems justifiable in a few of the cases in which deep seated tumors presented an isolated focus of true skin (Lecène,<sup>6</sup> Herrenschildt,<sup>41</sup> and Walther<sup>29</sup>).

The less formal genesis through metaplasia of the indigenous glandular epithelium is the more obvious explanation. Under pathological conditions the transformation of glandular to squamous epithelium has been demonstrated in tumors of the bronchi, gall-bladder, uterus, pancreas, stomach, rectum, prostate, and so on.

This explanation appears more rational, for most of the cases showed columnar and squamous epithelium side by side, and frequently transitions from the one to the other were apparent. Other factors which seem to favor this explanation are the physiological ability of the epithelium of the larger ducts to undergo squamous metaplasia during pregnancy, and the frequency with which squamous changes in the glandular epithelium are seen in diffuse fibromatosis and chronic cystic disease of the breast (Beneke,<sup>49</sup> Kuru,<sup>28</sup> Deaver and McFarland,<sup>38</sup> Dietrich and Fragenheim,<sup>50</sup> Biebl,<sup>35</sup> and Lee and Pack<sup>48</sup>).

Adeno-acanthoma does not require a multicentric origin from glandular and squamous epithelium *per se*, nor does it require an original heterotopia of squamous cells, but simply the neoplasia of gland cell carcinoma. This is a frequent characteristic of uterine tumors (Kaufmann,<sup>5</sup> and Ewing<sup>39</sup>).

#### SUMMARY

An intramammary tumor having a mixed structure of acanthoma, adenocarcinoma and sarcoma, is reported. The tumor had no demonstrable connection with the skin of the breast. The literature on squamous cell inclusions in intramammary tumors is reviewed.

NOTE: To Major Raymond O. Dart, Curator, and to Mr. R. M. Reeve, photographer, of the Army Medical Museum, we wish to express our indebtedness for the photographs.

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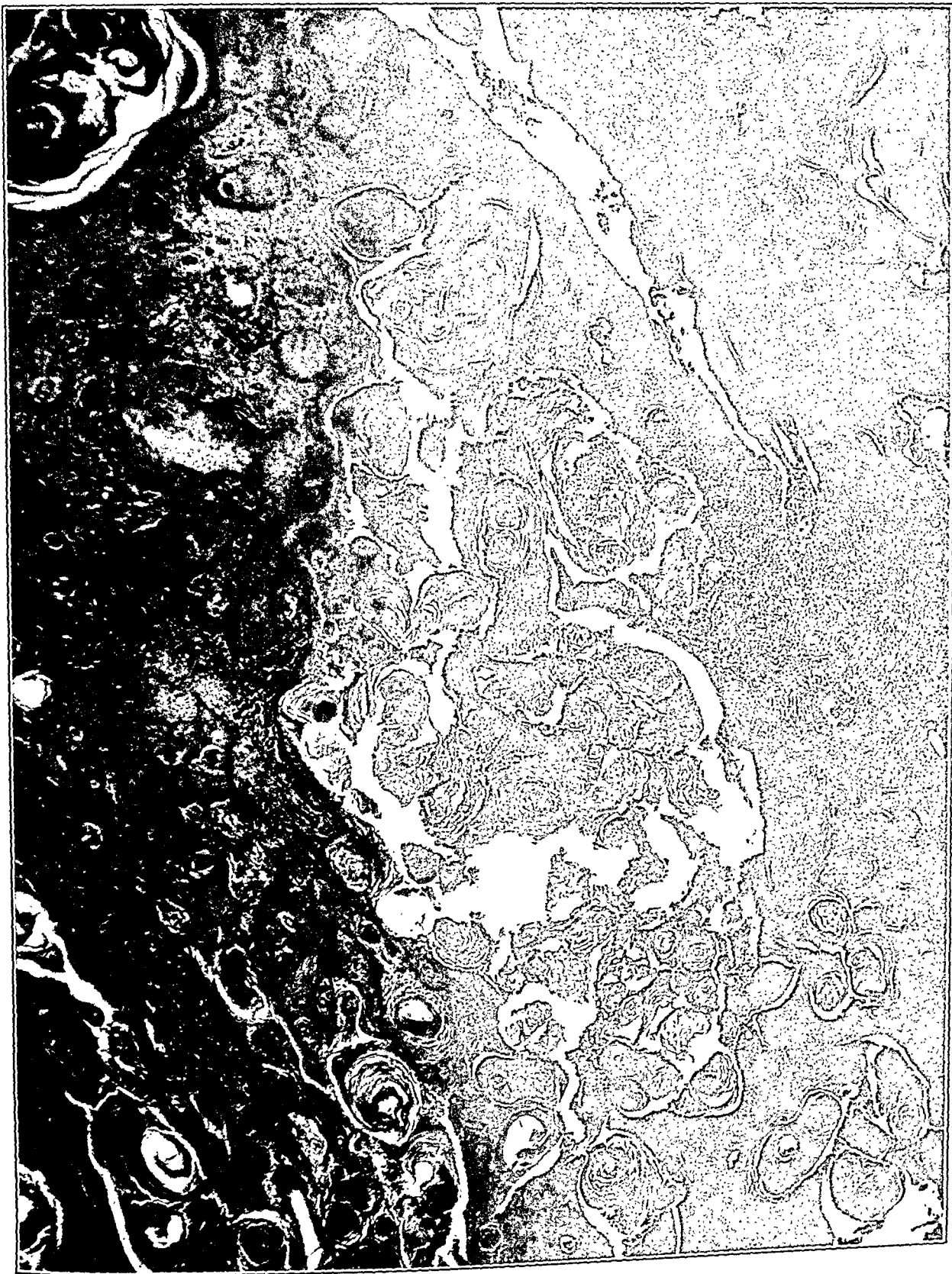
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## DESCRIPTION OF PLATES

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### PLATE 50

FIG. 1. Cysts filled with multiple pearl bodies (Army Medical Museum Acc. 64107).  $\times 35$ .



Pasternack and Wirth

Adeno-Acanthoma Sarcomatodes of Mammary Gland

PLATE 51

FIG. 2. An ancahomatous formation in the wall of a small cystic duct (Army Medical Museum Acc. 64106).  $\times 330$ .







# THE AMERICAN JOURNAL OF PATHOLOGY

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VOLUME XII

JULY, 1936

NUMBER 4

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## THE ORIGIN AND SIGNIFICANCE OF NEWLY FORMED LYMPH VESSELS IN CARCINOMATOUS PERITONEAL IMPLANTS \*

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It is the purpose of this paper to present the results of a study of the response of the lymphatics of the peritoneum to cancer cells which escape into the peritoneal cavity and often become implanted on its serous membrane. Special reference will be made to the origin and significance of newly formed lymph vessels in these metastases.

It has been clearly demonstrated, in a recent paper<sup>1</sup> by the writer, that the pathogenesis of these peritoneal metastases is but the attempted repair of injuries to the peritoneum caused by cancer cells which have escaped into the peritoneal cavity, lodged on the surface of its serous membrane and continued to grow in their new situation. The various stages in this process are similar to those encountered in the repair of tissues injured by foreign bodies and in the taking of skin grafts, namely the healing of wounds. The histological structure of these implants varies with the reaction of the peritoneum before and after the fixation of cancer cells, the activity of the latter and the age of the implants. As a result of these reparative processes carcinoma becomes embedded in the peritoneal scar, encapsulated on its surface, enmeshed in adhesions or, like a surgical skin graft, grows on the surface of the peritoneum without encapsulation. All stages in the pathogenesis of these lesions are illustrated in the above mentioned paper<sup>1</sup>: the escape of cancer cells

\* Received for publication April 3, 1936.

into the peritoneal cavity from the ovarian tumor, the presence of these cells in the ascitic fluid which usually accompanies this condition, the fixation of the cancer cells by fibrin on the surface of the peritoneum, and the other reactions of the injured peritoneal tissue manifested either by fibroblasts and other wandering cells alone or by these cells and newly formed blood capillaries which grow out through breaks in the mesothelium. The latter phase represents the granulation tissue stage of this type of implant which becomes transformed into a fully organized tumor with its permanent connective tissue stroma and blood vessels which are derived from its host.

Further studies, since the publication of the previous paper,<sup>1</sup> have taught me that in some instances a marked local proliferation of mesothelial cells may also occur in patients with peritoneal carcinomatosis and may play an important rôle in the pathogenesis of some implants. The cancer cells become surrounded by these proliferated cells which may form a part of the granulation tissue if the latter should develop. I have not been convinced that during the involution of the granulation tissue the mesothelial cells become converted into connective tissue cells. I do not know what happens to them.

The granulation tissue in which cancer cells become embedded presents a variety of forms depending on the reaction of the peritoneal tissues. In some instances it is scanty and low and at other times it is exuberant, as has been well shown in the previous paper<sup>1</sup> and will be illustrated again in the present study. The character of this tissue plays an important rôle in determining the form and structure of the mature implant.

The majority of the implants on the surface of the peritoneum, which result from the embedding of cancer cells in granulation tissue, are well suited for the study of the present problem since the greater portion of them, or even the entire metastasis, may jut beyond the surface of the serosa. The stroma including the vessels in the portion of the metastasis above the peritoneum must be newly formed. Many of these protruding implants resemble polyps which may be sessile with solid or fenestrated bases. In other instances the polyps may be attached to the peritoneum by one or more vascular pedicles which may be short or long, thick or slender. A study of these implantation metastases will convince one that they possess potentialities of invasion and dissemination similar to those of a

primary growth. If any of the carcinoma of the implant is exposed to the peritoneal cavity cancer cells may readily escape into that cavity and give rise to secondary implantations on the peritoneum. By the continuous extension of carcinoma in the base or pedicle of the implant the tissues of the host are invaded.

The lymph vessels of the host just beneath the base or pedicle of the implant may contain carcinoma which may be positively proved to be continuous with that in the peritoneal growth above it. In other instances this continuity cannot be established. I interpret both of these phenomena, in the majority of the cases which I have studied, as an indication of the invasion of these vessels by cancer cells from the nearby implant rather than evidence of retrograde lymphatic permeation or metastasis from the primary ovarian tumor and the origin of the peritoneal implant in this manner. In a few instances the histological picture suggested the presence of lymph vessels in the implant with carcinoma in them. These findings aroused my curiosity and induced me to study the problem presented in this paper.

#### THE PERITONEAL LYMPHATICS AND THEIR RELATION TO THE PERITONEAL CAVITY

The following descriptions of lymphatics, which might be of assistance in the study of this problem, are taken from Maximow.<sup>2</sup> "The lymphatic capillaries are thin-walled blindly ending tubes which form a dense network in most tissues of the body. . . . They have irregular shapes and are constricted in some places and dilated in others. . . . They branch abundantly and anastomose freely with one another. . . . Dilatations occur frequently where several capillaries join. . . . The lymphatic networks are often located beside networks of blood capillaries. . . . As a general rule, the lymphatic networks are farther from the surface of the skin or mucous membrane than the blood capillary networks. . . . Further, the lymphatic networks are distinguished from the blood capillaries by ending blindly in rounded or swollen ends. . . . The wall of the lymphatic capillaries is formed by a single layer of flat endothelial cells; these are slightly larger and thinner than those of the blood capillaries. . . . Each cell contains an oval flattened nucleus. . . . In sections of collapsed lymphatic capillaries, only the endothelial

nuclei can be seen and these cannot be distinguished from the nuclei of the surrounding fibroblasts. . . . The lymph passes from these capillary networks into lymphatic vessels which have slightly thicker walls and valves. . . . Although valves are not present in all lymphatic vessels, when they occur they are usually much closer together than those in veins."

According to Hertzler<sup>3</sup>: "The lymph vessels of the peritoneum are distributed everywhere, there being a detailed variation only in the arrangement and in the ease with which they are demonstrated. . . . They form a network of channels, beneath the basement membrane of the peritoneum in the same stratum that the blood vessels occupy, or more deeply, as von Recklinghausen believes. . . . The lymphatics of the visceral peritoneum in the intestinal tract unite with those of the mesentery. . . . Those over the solid parenchymatous organs unite with the lymph system of the organs, while those of the parietal peritoneum become a part of the lymph system of that region."

Many contributions have been made to the lymphatics of the various organs and structures on the peritoneal surfaces of which carcinoma may become implanted. However, it seems to me that too little attention has been paid to the lymphatics of the peritoneum itself in these situations. A knowledge of the distribution of the serosal and subserosal lymph capillaries in all portions of the peritoneal cavity, with special reference to their distance from the surface of the serosa and their relation to the blood capillaries, is of the greatest importance in the study of this problem. I have found Andersen's contribution<sup>4</sup> to the lymphatics of the Fallopian tube very helpful in the study of the origin of possible lymphatics in the stroma of carcinomatous implants on the surface of that organ. With the assistance of her illustrations I have been able to identify more definitely the non-injected serosal lymphatics of the tube in my sections.

It has been demonstrated by many workers that small particulate matter such as lamp black and cinnabar, which has been introduced into the peritoneal cavity of lower animals, soon appears in the lymph vessels and lymph nodes above the diaphragm of these animals.

Drinker and Field<sup>5</sup> recently have given an excellent presentation of the lymphatics draining the peritoneal sac. According to them,

“von Recklinghausen, in 1862, called attention to the diaphragm as the most important path for absorption of intraperitoneal foreign particles.” “Casparis,” they say, “has shown that omental lymphatics are responsible for a small fraction of particle absorption from the peritoneal cavity.” They state: “It is generally agreed that the parietal peritoneum is of little importance in dealing with particles and that the mesenteric peritoneal surface is also unimportant.” These authors also note that “the size of particles taken into lymphatics by the diaphragmatic route may be very large, the red cells of birds being taken up rapidly when injected into the peritoneal sac of mammals.”

MacCallum <sup>6</sup> has shown that there are no actual communications between the peritoneal cavity and the lumina of the lymphatics of the diaphragm. On the other hand, he has demonstrated that phagocytes laden with pigment escape from the peritoneal cavity into the diaphragmatic lymphatics by passing between the mesothelial cells covering the diaphragm and also between the endothelial cells lining the lymph channels. Through a similar route free particles in the peritoneal cavity are aspirated into these lymphatics by the mechanical force of the respiratory movements which during inspiration causes a momentary separation of the cells covering the diaphragm and those lining its lymphatics.

Cunningham <sup>7</sup> believes that this material passes through the living protoplasm of the mesothelial and endothelial cells rather than between these cells.

Florey, <sup>8</sup> as a result of similar studies, presents evidence that intra-abdominal pressure is the factor controlling the rapid absorption of fluids and particles by way of the diaphragmatic lymphatics. His illustrations show the passage of graphite ink out of the peritoneal cavity between the mesothelial cells and not through their protoplasm.

The abdominal cavity of patients with peritoneal carcinomatosis is usually distended with fluid containing in suspension particulate matter, namely cancer cells, which have escaped into this cavity. Is it possible that the distention caused by this fluid might separate some of the mesothelial cells covering the peritoneum of the abdominal walls and diaphragm and that increased intra-abdominal pressure might force cancer cells, suspended in the ascitic fluid, through the resulting gaps between the mesothelial cells into the

peritoneal tissues? Carcinoma is found in the serosal lymph vessels of patients with peritoneal carcinomatosis. Pleuritic carcinomatosis secondary to peritoneal carcinomatosis occurs but it is an unusual complication in the early stages of the latter condition. Carcinoma implanted on the under surface of the diaphragm may invade this structure and appear on its upper surface in the same manner that the neoplasm in any implant invades the tissues of its host. It seems unlikely to me that cancer cells, so often present in the ascitic fluid of these patients, could gain access to the lumina of the serosal lymph vessels by passing between or through the mesothelial cells covering the surface of the peritoneum and the endothelial cells lining the peritoneal lymphatics as the red blood corpuscles of birds and particles of lamp black and cinnabar introduced in the peritoneal cavities of mammals reach the diaphragmatic lymph channels. The cancer cells in ascitic fluid usually occur in clumps. The size of these clumps should prevent their ready passage into the serosal lymph channels. The reaction of peritoneal tissues towards cancer cells may interfere with the permeability of these tissues. The way that cancer cells, floating about in ascitic fluid, actually reach the lumina of the peritoneal lymph vessels will be presented later in this paper.

#### THE STATUS OF THE RESPONSE OF LYMPHATICS TO INJURY AND OF THE PRESENCE OF NEWLY FORMED LYMPH VESSELS IN GRANULATION TISSUE, ADHESIONS AND NEOPLASMS

Reichert,<sup>9</sup> in 1926, published the results of a series of experiments demonstrating the remarkable regenerative ability of severed lymphatics following the replanting of the hind legs of dogs after a circular incision through the middle third of the thigh, leaving only the femoral artery and vein and the femur. The extremity, thus nearly amputated, was replanted by a careful approximation of the muscle stumps and skin edges with silk sutures. At varying intervals, of from two days to fourteen months after the operation, diluted India ink was injected into the foot pads and skin of the leg and thigh distal to the operative line and also into the popliteal lymph node. Several hours later the animals were killed with ether. In some instances the arterial system was also injected with a white bismuth mass. New lymphatics were shown to have crossed the

scar of the incision as early as the fourth day. By the eighth day the regeneration was physiologically adequate in both the deep and the superficial sets of lymphatics. Concurrent experiments showed that compensatory arterial and venous regeneration occurred by the third and fourth days respectively.

Clark and Clark,<sup>10</sup> in 1932, demonstrated the new growth of lymph vessels in the rabbit's ear. By means of transparent chambers inserted in the ears of this animal they were able to study, under the microscope, the growth of lymph vessels as well as blood vessels and other tissues present in the observation area. All structures in this area were newly formed and had invaded the hole which had been cut through the ear at the time of the installation of the chamber. By this device they were able to observe the growth of the same living vessels over a period of several weeks. According to their observations the lymphatics are more sluggish than blood vessels in their reactions. The growth of new lymphatics is always subsequent to that of the blood vessels by days, weeks or months. They send out fewer sprouts, form fewer anastomoses and are more easily blocked in their advance but, once formed, they are more stable and more persistent than the new blood capillaries.

McMaster and Hudack,<sup>11</sup> in 1934, demonstrated the response of the lymphatics of the skin of the ears of mice to incisions and burns by injecting the lymphatics of the ear at varying intervals after the injury. Occasionally on the sixth and seventh days and characteristically from the eighth day on, after the incision of the skin of the ear, evidence of new formation of lymphatics was observed. They found that lymphatic drainage might be instituted from areas of repair before the drainage of the blood vessels. Thus, severed lymphatics seemed to have reunited before there was evidence of functioning blood vessels. Great numbers of new lymphatics were seen by them in the areas recovering from burns. When the latter had been severe enough to cause necrosis and perforation of the ear many tiny lymph capillaries were found in the granulation tissue of the healing margins, obviously taking their place in actively growing tissue.

Coffin,<sup>12</sup> in 1906, twenty-eight years before McMaster and Hudack's <sup>11</sup> work, demonstrated the growth of lymphatics in granulation tissue but did not mention in his report the kind of animal used. A loop of intestine was brought outside of the abdominal



wall. Ten days later the lymphatics of this loop were injected. Injected lymph vessels could be seen in the granulation tissue which had developed on the exposed surface of the intestine. By means of serial sections through the intestinal wall and overlying granulation tissue he found that the original lymphatics of the wall were filled with the injection mass and that sprouts had arisen from these and had invaded the granulation tissue.

Poirier,<sup>13</sup> in 1890, described very accurately the injection of lymphatics in adhesions which extended from the posterior surface of the uterus to the rectum and the pelvic wall. He observed that when he punctured very superficially the surface of the uterus, as in injecting the serosal lymphatics, the lymphatics in a small area of the peritoneum were filled. At the same time the injection mass (mercury) invaded the lymphatics of the adhesions and from these extended to large lymph vessels of the rectum and pelvic wall and even reached neighboring lymph nodes. He believed that the lymphatics in these adhesions arose from a sprouting and outgrowth of the lymphatics of the uterine serosa, caused by a prolonged inflammation of uterine origin. It would seem to me that the presence of lymphatics in these adhesions was due to the formation and persistence of similar vessels in granulation tissue which preceded the adhesions and from which the latter were derived.

Manasse,<sup>14</sup> in 1894, described what he believed to be lymph vessels in polypoid granulation tissue on the tympanic membrane from a patient with chronic purulent otitis media. He states that these vessels terminated with patent lumina on the surface of this granulation tissue. From his description of these channels as well as from his illustrations I am not fully convinced that they were lymph vessels.

Talke,<sup>15</sup> in 1902, found newly formed lymph vessels accompanying blood vessels in adhesions about the ascending colon. He also described various types of newly formed lymph vessels in organized pleuritic membranes. He was able to establish the continuity of some of these vessels with preëxisting lymphatics in the pulmonary pleura beneath the membrane. Unfortunately there are no illustrations in his paper. However, I believe that at least some of the channels described by Talke were lymph vessels.

The above mentioned experiments and observations have an important bearing on the solution of the problem presented in this

paper. Since lymphatics respond to operative trauma and are regenerated in the same manner and in some instances as quickly as blood vessels, we might infer that newly formed lymphatics might be present in the stroma of some implantation peritoneal metastases containing newly formed blood vessels. However, this might be possible only when lymph capillaries accompany the blood capillaries of the host from which the newly formed blood vessels of the tumor are derived. When the lymph capillaries are more deeply situated below the surface of the peritoneum, as is apparently a frequent occurrence, they might be out of range of the traumatic or stimulating agent causing the outgrowth of the endothelial cells of the blood capillaries.

It has been shown that newly formed lymphatics arise in granulation tissue from preëxisting lymphatics in lower animals and have been found in peritoneal and pleuritic adhesions in human beings. If present in adhesions they also should have been present in the granulation tissue from which the adhesions were derived. Granulation tissue plays a very important rôle in the development of many of the implantation metastases on the peritoneum. It forms the foundation (base or pedicle), the framework (stroma) including the blood vessels, and the walls and roof (capsule) of many of these metastases. In fact it forms the entire tumor except the cancer cells.

Implantations of cancer frequently occur on the surface of the intestine. Since it has been shown by Coffin<sup>12</sup> that newly formed lymphatics develop in experimentally produced granulation tissue on the surface of the intestine we may infer that they might sometimes arise in granulation tissue caused by the lodgment of cancer cells on the surface of the intestine of human beings, if the distribution of the lymphatics is the same in both instances. We likewise may infer that newly formed lymphatics might sometimes occur in the stroma of other neoplasms containing newly formed blood vessels.

Lee and Tilghman<sup>16</sup> in a recent paper reported the results of the intratesticular inoculation of ten rabbits with a suspension of transplantable rabbit carcinoma and they presented a careful study of the relation between the lymph vessels of the testis and cancer masses in that organ. They also reviewed the literature on the lymphatics of malignant tumors and criticized observations made by Krause<sup>17</sup> and by Evans.<sup>18</sup> At varying intervals, after artificial implantation

had been produced, the lymph vessels of the testis were injected with India ink or a saturated solution of Berlin blue. In five of the animals the blood vessels were also injected. They were unable to demonstrate any lymphatics in the cancer masses. The only situation in which lymph vessels were found in close proximity to carcinoma was where this growth was invading normal testicular tissue containing preëxisting lymphatics. They have given an excellent description of the carcinomatous invasion and subsequent destruction of these lymphatics.

Lee and Tilghman<sup>16</sup> made serial sections of a small nodule of carcinoma, about 4 mm. in diameter, attached to the surface of the tunica vaginalis propria of the testis. The lymph vessels of the tunica vaginalis beneath the nodule were well injected but branches from these did not extend into the nodule. The writers believed that this nodule arose from the growth of cancer cells which had become implanted on the surface of the tunica. They state that it was common to see such isolated cancer nodules throughout the entire peritoneal cavity. These writers compare the results of their study of the lymphatics of implantation carcinoma on the peritoneum of the rabbit with a similar study made by Goldmann<sup>19</sup> in mice.

The latter worker studied both the blood and lymph supply of mouse carcinoma transplanted into the peritoneal cavity. To demonstrate the lymphatics he injected ink into the lymph vessels of the loop of the intestine on the surface of which a metastasis was present. The injections were made only into the submucosa as this layer is apparently the only one in the intestinal wall of the mouse that has a lymph plexus. He showed that a tumor mass which had become attached to the surface of the intestine received an abundant blood supply from its host but in no instance were any lymph vessels seen extending from the intestinal wall to the metastasis.

The observations of Goldmann<sup>19</sup> were in entire accord with those reported by Lee and Tilghman.<sup>16</sup> The latter call attention to an interesting and, I believe, a very important difference in the two sets of experiments. In the mouse the lymphatic plexus nearest the cancer was separated from it by two layers, the muscular and serous coats, whereas in the rabbit the cancer was situated directly in the serous coat into the lymph vessels of which injections were made. Apparently even the greater proximity of lymph vessels and cancer

did not facilitate any lymphatic extension into the carcinoma. Lee and Tilghman<sup>16</sup> in their description of the microscopic examination of the nodule of cancer implanted on the tunica vaginalis of the rabbit's testicle state: "The scant blood supply to this mass was derived from a few small vessels which passed through the delicate areolar tissue connecting the nodule to the testis. . . . The small tumor was quite compact, in spite of the numerous areas of necrosis; no loose areolar tissue surrounded the blood vessels." From my own studies I would not expect to find lymphatics in the nodule described by them. I have been able to detect them only in the stroma of metastases either with many blood vessels in it or in stroma in which I judged that at one time (during the granulation tissue stage) the blood vessels had been numerous: I have not observed them in all of these. The lymphatics usually accompany the blood vessels and cannot be detected when their walls are compressed. The metastasis described by Goldmann<sup>19</sup> had an abundant blood supply but, as pointed out by Lee and Tilghman,<sup>16</sup> the lymphatic plexus of the intestinal wall of the mouse, which had been injected, was separated from the metastasis by the muscular and serous coats.

Krause,<sup>17</sup> in 1863, by injecting the lymphatics of the skin adjacent to tumors demonstrated the presence of lymphatics in the tumors and showed that they were continuous with those in the skin. Lee and Tilghman<sup>16</sup> reviewed the work of Krause and believed that it was quite possible that these lymphatics "were in normal tissue which was about to be invaded by the cancer growth."

Evans,<sup>18</sup> in 1908, by injecting the lymphatics in the region adjacent to a metastatic sarcomatous nodule in the wall of the intestine demonstrated lymph channels which were continuous with pre-existing and neighboring lymph vessels. Lee and Tilghman<sup>16</sup> quote MacCallum as believing that Evans did not make it clear that the injected lymph vessels were actually newly formed in the tumor and not merely distorted and dislocated lymphatics which were originally situated in the wall of the intestine. In their conclusions they stated: "Examination of the literature makes it doubtful whether any true newly formed lymph vessels were ever observed in cancer tissue; and transplantable rabbit carcinoma does not have any lymph vessels."

## MATERIAL AND METHODS OF STUDY

The material for this problem was obtained from the gynecological service of the Albany Hospital. The work was carried on in the pathological laboratory of that hospital and the Albany Medical College.

I wish to emphasize the importance of the following procedures: careful inspection of conditions present at the time of the operation; prompt removal of interesting bits of tissue and the immediate placing of such material in a fixing solution; the making of sketches of gross specimens before they are sent to the laboratory; and the blocking of all tissue and the studying of the sections personally. In our laboratory the embedding of blocks of tissue and section cutting and staining are entrusted to a well trained technician, Miss Helen Buchan, who studies the gross specimen with me. She is present when the blocks are made and understands what may be expected in each block. She is not only skilled in making serial celloidin sections but is also able to recognize under the microscope the different structures encountered in these sections, so that she can decide when serial sections are indicated.

All of the tissue from which the sections shown in this paper were made was fixed in 10 per cent formalin and embedded in celloidin. I believe that this procedure lessens unequal tissue shrinkage and, therefore, fewer distortions and exaggerated tissue spaces occur than in paraffin embedding after formalin fixation. Since we often fix large pieces of tissue, sometimes the entire specimen, we have found formalin and celloidin better adapted to our needs. The sections were stained with hematoxylin and eosin.

Serial sections are extensively used and are sometimes indispensable. In some instances all sections are saved, stained and studied, but more often every third or sixth section, according to the judged importance of the tissue, is stained and examined. Often a section is stained while cutting the block in order to ascertain better the exact condition present. If this section proves to be interesting serial sections are prepared. Otherwise, since serial sections, even when interrupted, are time consuming and frequently unfruitful, they are not made.

I fully realize the great difficulties which are encountered in studying lymphatics which are not injected and the inaccuracies

which must result. The nuclei of their lining endothelial cells, for example, cannot always be distinguished from those of fibroblasts. Lymph vessels, especially small ones, with collapsed walls cannot be detected. Patent tissue spaces may sometimes be mistaken for lymph vessels, especially if, as too often happens, they are exaggerated by the improper fixation and embedding of the tissue. Lymph vessels may also simulate empty veins.

I have studied the nuclei of the endothelial cells lining unquestionable lymph vessels and have learned that they do not always appear to be uniformly spaced and do not seem to be the same size in all lymphatics. In addition, the appearance of these oval flattened nuclei varies with the angle at which they are viewed or cut in the section.

Additional difficulties are encountered in the study of possible lymph vessels in granulation tissue on the surface of the peritoneum. These vessels may be confused with spaces arising from the incomplete fusing of strands or different portions of granulation tissue or with spaces caused by the arching of granulation tissue over a portion of the surface of uninjured peritoneum. The latter condition is not as troublesome as the former because such spaces are usually partially or entirely lined by mesothelial cells which usually can be recognized.

I believe that lymph vessels in granulation tissue usually accompany blood capillaries in the development of this tissue. When in granulation tissue a vessel, either empty or containing a few lymphocytes and lined by endothelium-like cells, accompanies blood vessels which are filled with blood, this structure is probably a lymph vessel. If it can be traced, even with interruptions, towards a recognizable preëxisting peritoneal lymph vessel its identity is more certain. The only positive identification is to establish this continuity. Although this continuity may exist it cannot always be detected, even in complete serial sections, because if the walls of a portion of the vessel be collapsed, this portion of the vessel cannot be distinguished from the tissues surrounding it. It is sometimes impossible to detect any lymph vessels in the peritoneum just beneath the granulation tissue. This in many instances may be due to the compression of these vessels by the tissue surrounding them. It is noted in some instances that, when the vessels are dilated and easily seen, the peritoneal tissue is relatively loose and apparently edematous. Pullinger and Florey<sup>20</sup> have shown that lymph vessels

are intimately connected with the tissue surrounding them and that this connection is responsible for the dilatation of the vessels seen in the localized edema experimentally produced by these workers.

I shall present some of my observations in the study of this material and shall give my interpretation of these. I fully realize that, due to technical difficulties, some of these interpretations may be erroneous. I desire others to draw their own conclusions, not only from the conditions portrayed in the photomicrographs of this paper but, of much greater importance, from the study of similar material of their own in which, if possible, the lymph vessels have been injected.

### THE SOLUTION OF THIS PROBLEM

The polypoid implants are the ones that are best suited for the study of this problem since the portion of the implant that juts above the surface of the peritoneum must be newly formed and is easily seen. It is true that all of this tissue, except the cancer cells, is derived from the peritoneum. Furthermore, carcinoma is responsible for the development of the tissue that forms the stroma of the implant. A given polypoid implant may arise in two ways. In one, an exuberant growth of granulation tissue develops about cancer cells which have lodged on the surface of the peritoneum. In the other, cancer cells lodge on and become enmeshed in growing granulation tissue which is often present in these cases. Carcinoma is not always present in all portions of this tissue. If cancer cells become embedded in a portion of this tissue which may or may not contain carcinoma it may be claimed that they have become implanted in preëxisting newly formed tissue which has not been caused by these particular cancer cells. I believe that that is a quibble since the granulation tissue in which the cells are implanted was caused by carcinoma and will continue to develop and form the stroma of any neoplasm growing in it.

Polypoid implants are of frequent occurrence and present a variety of forms, all stages in the life histories of which may often be found in the material obtained from one patient. The developmental history of these implants is that of granulation tissue which arises on the surface of the peritoneum and is modified by the presence of a growing and invading foreign body, namely, the carcinoma which caused the granulation tissue to form in the first place.

We must first consider the life history of granulation tissue and that of its component elements. Excellent descriptions of this tissue may be found in the standard textbooks of pathology including those by Adami,<sup>21</sup> Aschoff,<sup>22</sup> Bell,<sup>23</sup> Borst,<sup>24</sup> Boyd,<sup>25</sup> Delafield and Prudden,<sup>26</sup> Green,<sup>27</sup> Karsner,<sup>28</sup> Kaufmann,<sup>29</sup> MacCallum,<sup>30</sup> Mallory,<sup>31</sup> Muir,<sup>32</sup> Ribbert,<sup>33</sup> and Ziegler.<sup>34</sup> Only three of these authors mention the presence of lymphatics in granulation tissue: Delafield and Prudden base their statement on the work of Talke,<sup>15</sup> Karsner on that of McMaster and Hudack,<sup>11</sup> and MacCallum on that of Coffin.<sup>12</sup> Since Coffin,<sup>12</sup> Clark and Clark,<sup>10</sup> and also McMaster and Hudack<sup>11</sup> have found lymph vessels in granulation tissue experimentally produced in animals we would expect to find them in granulation tissue that results from injury and disease in human beings. The demonstration of lymph vessels in peritoneal adhesions by Poirier<sup>13</sup> and in both peritoneal and pleuritic adhesions by Talke<sup>15</sup> indicates that they might have been present in the granulation tissue that preceded the adhesions. It is evident that if lymph vessels occur in granulation tissue in human beings and are of any scientific interest and clinical importance they deserve a greater recognition than has been accorded them in the past.

The entire life history of granulation tissue is interesting, not only its origin and development but also its retrogression or involution after the removal of the exciting cause. In this problem we are especially concerned with the origin of its blood vessels and the fate of the overabundant supply of these, because if lymph vessels are present we would expect them frequently to accompany the newly formed blood channels. Because of involutionary changes in the blood vessels, as well as pressure exerted by the increasing density of the surrounding tissue, many or even all of these vascular channels may disappear. If lymph vessels are present in granulation tissue their very structure is such that they might be even less able than blood vessels to resist involutionary changes and pressure exerted by surrounding tissues. However, Clark and Clark<sup>10</sup> believe that they are more stable and persistent than the newly formed blood vessels in this tissue.

Granulation tissue arising on the surface of the peritoneum has a life history similar to that of granulation tissue elsewhere except as it may be modified by the structure of the peritoneum. All that may remain of an exuberant growth of this tissue on the peritoneum is a



slight local peritoneal thickening and this is not always present. In other instances adhesions result but in time even these may disappear.

When cancer cells are present in granulation tissue on the surface of the peritoneum the exciting cause of this tissue remains. This results in a persistence of the stroma and blood supply of the new growth. Therefore the granulation tissue stage of the implant is prolonged, thus deferring or even preventing the complete involution of this tissue.

Granulation tissue without demonstrable cancer cells in all parts of it is frequently present on the surface of the peritoneum in patients with peritoneal carcinomatosis. This condition is more apt to be found early in the disease but may be observed in any stage. It appears that sometimes cancer cells escaping into the peritoneal cavity may cause local serosal reactions without being demonstrable in all portions of the resulting granulation tissue.

Granulation tissue with carcinoma in it differs from that without it in two respects—first, in the presence of the tumor, and second in the distortions caused by the growing neoplastic cells. It is obvious that if lymph vessels occur in granulation tissue without cancer cells in it they must also be present in nearby similar tissue containing carcinoma, even though they may not be recognized as such because of the compression or occlusion of their lumina by the neoplasm. If lymphatics are not found in cancer-free granulation tissue from patients with peritoneal carcinomatosis they probably are not present in similar tissue containing carcinoma. Therefore, if we can demonstrate the presence of lymph vessels in cancer-free granulation tissue from these patients we may reasonably conclude that they are also present in the implants themselves, the stroma of which has been derived from this tissue.

I shall first demonstrate newly formed lymph vessels in cancer-free granulation tissue on the surface of the peritoneum of patients with peritoneal carcinomatosis. Structures which I believe to be newly formed lymph vessels have been found in such granulation tissue on the parietal peritoneum and on the peritoneum covering the appendix, Fallopian tubes, uterus, omentum and epiploic appendages. I have never seen them in granulation tissue on the surface of the ovary or in implants arising in this situation.

## CASE REPORTS

The illustrations with their legends present my observations and interpretations better than any written description alone. The purpose of the brief reviews of the following cases is to coördinate and supplement these.

CASE 1. The patient, A. H. No. 9272-34, had a large adenocarcinoma of the left ovary, which had extended through the surface of that organ and had possibly superficially invaded the mesentery of the small intestine which was adherent to the tumor. Turbid fluid was present in the peritoneal cavity. Cancer cells were found in the sediment obtained by centrifugalizing this fluid. An early peritoneal carcinomatosis was found on the posterior surface of the right uterine cornu and on the surface of the adjacent ovary. The patch of cancer-free granulation tissue on the parietal peritoneum, shown in Figures 1 to 12 inclusive, was situated lateral to the abdominal incision and directly over the ovarian tumor. In the illustrations it is important to note that newly formed lymph vessels are present in this granulation tissue. They arise from preëxisting lymph vessels in the underlying peritoneum and with the newly formed blood vessels extend into the tufts of granulation tissue. In some instances they reach the very tips of these tufts. These newly formed lymph vessels are situated in granulation tissue caused by carcinoma, the very tissue that forms the stroma of all polypoid implants as well as that of many of the other types. In this study of one small patch of granulation tissue on the parietal peritoneum the first step in the solution of our problem has been attained because the sections show that lymphatics have grown from preëxisting lymph vessels into this newly formed granulation tissue. Furthermore, the findings in this granulation tissue indicate that newly formed lymph vessels sometimes may occur in similar tissue containing cancer cells and in the stroma of mature implants. Newly formed lymph vessels also may be present in the stroma of some primary neoplasms containing newly formed blood vessels. It is evident that when newly formed lymph vessels occur in the stroma of any carcinoma an early lymphatic dissemination of the growth is more possible than when they are not present.

The granulation tissue on the posterior surface of the right uterine cornu is evidently of more recent origin than that just described (see

Figs. 13 to 21 inclusive). Long strands of this tissue arise from the peritoneum and are arranged in varied and fantastic shapes. Cancer cells have evidently been caught in these strands like flies in a spider's web, and various stages in their implantation are present. These strands are very vascular and in many instances contain blood vessels which are accompanied by vessels or channels lined by endothelium-like cells. These possible lymph vessels can be followed, with interruptions, to similar vessels in the portions of the strands attached to the peritoneum. It is impossible to trace the vessels in the base of the granulation tissue strands to preëxisting vessels in the peritoneum beneath them since lymph vessels cannot be detected in this portion of the peritoneum. Although they may be present in the peritoneum they may not be recognized on account of the compression of their walls by the denser tissue in this situation. I realize that at least some of the lymph vessel-like structures in these strands of granulation tissue may be spaces created by the incomplete fusion of adjacent portions of this rapidly growing tissue. However, I doubt if this theory of origin can explain the histogenesis of all the spaces. The strongest points in favor of the lymphatic character of some of these possible lymph vessels are: they accompany newly formed blood vessels and in structure they are indistinguishable from proved lymph vessels in the granulation tissue on the parietal peritoneum from the same patient. If this concept be true newly formed lymph as well as blood vessels may be said to be present in the tissues in which carcinoma is embedded.

CASE 2. The patient, A. H. No. 88548, had a papillary adenocarcinoma of both ovaries and a similar carcinoma of the mucosa of the body of the uterus, associated with a widespread peritoneal reaction (granulation tissue) and ascites. Occasional clumps of cancer cells were found in this granulation tissue. Fully organized implants, however, were not detected. Certain features of this case have been published in two previous papers<sup>1,35</sup> (see Cases 6 and 4 of these).

In the present paper photomicrographs of granulation tissue on opposite sides of the same Fallopian tube are shown. Judged newly formed lymph vessels are present in the granulation tissue in both situations. Cancer cells are not found in one patch of granulation tissue but are present in the other (see Figs. 22 to 25 inclusive). If we may believe the evidence already presented we may infer that

the stroma of the fully organized implants resulting from these lesions should contain newly formed lymph vessels as well as newly formed blood vessels.

CASE 3. The patient, A. H. No. 6293, had adenocarcinoma of both ovaries associated with an extensive peritoneal carcinomatosis. A large amount of ascitic fluid was present. An exploratory incision was made. The ovarian tumors were so firmly attached to the surrounding pelvic structures that it was deemed inadvisable to attempt to remove them. Small pieces of the peritoneum with implantations on its surface and also a small portion of the lower border of the omentum were carefully removed and immediately placed in formalin. A small pedunculated polypoid implant attached to the vesico-uterine reflection of peritoneum proved to be most interesting and is presented in Figures 26 to 32 inclusive. The stroma of this implant is abundant and in many places vascular. The newly formed blood vessels are frequently accompanied by channels or vessels lined by endothelium-like cells and without any blood in their lumina. These judged lymph vessels are most evident in the stroma of the hilum of the kidney shaped tumor and in the opposite portion of its cortex. They can be traced from the cortex nearly to the midportion of the tumor. Here they are lost but seem to appear again in the stroma of the hilum of the implant. I believe that, at one time, they may have been present in the center of the tumor. At present they are not apparent in this situation, either because of the obliteration of their lumina by the denser tissue in this portion of the stroma or by carcinoma compressing or destroying them. While carcinoma is found in close proximity to some of these lymph vessels actual invasion of their lumina is not observed. Lymph vessels accompany newly formed blood vessels in the pedicle of the implant as well as the preëxisting blood vessels in the base of the pedicle from which the newly formed blood vessels of the implant are derived. From the above evidence I have concluded that this implant is supplied with newly formed lymph vessels which accompanied or followed the newly formed blood vessels in the development of the stroma of the tumor. The study of the non-polypoid implants on the parietal peritoneum and omentum also shows newly formed lymph vessels in the newly formed tissue in which the carcinoma is embedded (see Figs. 33 to 38 inclusive).

CASE 4. The patient, A. H. No. 3679-35, had an adenocarcinoma

of both ovaries and an associated peritoneal carcinomatosis. A large amount of ascitic fluid was present. Clumps of cancer cells were found in the sediment obtained by centrifugalizing this fluid. At the onset of the operation a small strip of the uterovesical reflection of peritoneum, including a small superficial portion of the adjacent anterior uterine wall, was carefully removed and placed in formalin in order that the delicate implants on its surface might not be injured during the subsequent course of the operation. The uterus and both tubes and ovaries were then removed. All of the photomicrographs from this case which are shown in this paper were made from sections of the small piece of tissue just mentioned.

Early granulation tissue, with cancer cells embedded in it, is present on the surface of the anterior wall of the uterus just above the attachment of the uterovesical reflection of peritoneum. Cancer cells are being added to the surface of this tissue. Newly formed blood vessels have extended into the granulation tissue through breaks in the mesothelial covering of the peritoneum. In one portion of this tissue the newly formed blood vessels are accompanied by a judged newly formed lymph vessel which can be traced back to a preëxisting lymph vessel in the peritoneum. We may infer, therefore, that if the sessile implant had matured its stroma would have contained newly formed lymph as well as blood vessels (see Figs. 39 to 48 inclusive).

A variety of pedunculated polypoid implants also are present. Sections of some of these show possible newly formed lymph vessels (see Figs. 52 to 72 inclusive). Their continuity with preëxisting lymph vessels in the peritoneum beneath the base of their pedicles cannot be established. This may be due to the inability to detect non-injected lymph vessels when their lumina are obliterated by the pressure of the tissues about them. However, in one implant with many pedicles judged newly formed lymph vessels are present in its stroma below the advancing carcinoma and also in all of its pedicles (see Figs. 63 to 72 inclusive). The continuity of the lymph vessels in the pedicles with preëxisting vessels in the peritoneum beneath their bases is very strongly indicated but cannot be definitely proved. The invasion of a preëxisting peritoneal lymph vessel by carcinoma can be seen in the advancing edge of a large sessile polypoid implant (see Figs. 73 to 76 inclusive). The invasion of a possible newly formed lymph vessel can also be seen in the less dense vascular tissue

of the base and advancing edges of the same implant (see Figs. 80 and 81). Newly formed lymph vessels cannot be detected in the dense mass of carcinoma forming the greater portion of the implant.

The judged extension of lymph vessels into low granulation tissue on the surface of the peritoneum is present in a few situations (see Figs. 114 to 117 inclusive, and also Fig. 124).

CASE 5. The patient, A. H. No. 7467-30, had carcinoma of both ovaries, associated with an extensive peritoneal carcinomatosis. Both ovaries were adherent to the sides of the pelvis and posterior uterine wall with evidence that the ovarian tumors had invaded these structures. Implantations in various stages of development were present on the surfaces of the ovaries, the uterus, Fallopian tubes and epiploic appendages. The earlier stages of these lesions are seen at their best on the surfaces of two epiploic appendages.

Sections show clumps of cancer cells in the sediment obtained from the centrifugalized ascitic fluid (Fig. 82), in the lumina of both tubes and in the lymph vessels of the ovaries and the walls of the uterus and the tubes. The arrangement of the cancer cells is the same in all of these situations and is also similar to that in the ovarian tumors and in the metastases. All stages in the development of these metastases are present on the surfaces of two epiploic appendages, yet carcinoma cannot be demonstrated in lymphatics of these structures (see Fig. 83). These peritoneal metastases are histologically identical with those on the surfaces of the tubes beneath some of which carcinoma is found in the lymph vessels of the tubal wall. Carcinoma is found replacing the mucosa of one of the tubes in two places. It is interesting that these are the only situations in which carcinoma is also found in the lymphatics of the mucosa of either tube. As previously stated, clumps of cancer cells are abundant in the lumina of both tubes. The relation between the cancer cells in the implants and those in the underlying lymphatics is an interesting problem. I am sure that this phenomenon would be considered by many pathologists a manifestation of retrograde lymphatic permeation and metastasis from the ovarian tumors. Some might even believe that the serosal implantations (metastases) in this case arose in this manner. The correctness of such an interpretation cannot be positively refuted. However, I believe that cancer cells in the ascitic fluid became enmeshed on the serosa of the Fallopian tubes as on the serosa of the epiploic appendages and like

primary tumors invaded the underlying structures. It is also quite possible that newly formed lymphatics in these implantations may have aided in the escape of cancer cells from the implants into the preëxisting lymph vessels of the tubal wall. The reasons for these interpretations are brought out in Figures 84 to 95 inclusive with their legends.

CASE 6. The patient, A. H. No. 113036, had a large adenocarcinoma of the right ovary associated with an extensive peritoneal carcinomatosis. Photomicrographs are shown of sections from two sessile polypoid implants on the surface of the mesoappendix at its attachment to the appendix. Carcinoma has invaded the preëxisting tissues of their host beneath these implants. Of particular interest is the response of the peritoneal lymphatics of the appendix about the advancing margin of one of these implants (see Figs. 96 to 98 inclusive). In this situation low granulation tissue has developed, in which dilated lymph vessels are present. These vessels arise from preëxisting vessels which either expand in the newly formed tissue or actually grow out into it. All stages in the development of these dilated lymph vessels are present. One can follow the gradually increasing response of these lymph vessels as one approaches the margin of the implant where the reaction is most marked. Cancer emboli are found in some of these dilated lymph vessels. If newly formed lymph vessels are present in the implant proper they are either compressed or invaded by the carcinoma so that they cannot be recognized. It is quite possible that they are there and that the carcinomata in the lymphatics just described have reached their present situation through newly formed lymph vessels in the granulation tissue stroma about the advancing margin of the implant. This is strongly suggested but cannot be definitely proved because serial sections were not made of these implants.

CASE 7. The patient, A. H. No. 8025-33, had an adenocarcinoma of both ovaries associated with extensive peritoneal carcinomatosis. A large amount of ascitic fluid was present. Clumps of cancer cells were found in the centrifugalized sediment obtained from this fluid. A small implant on the surface of one of the Fallopian tubes was most interesting. A section of this implant (Fig. 102) illustrates the early granulation tissue stage in the development of a sessile polypoid implant. Clumps of cancer cells are embedded in this granula-

tion tissue which forms the stroma of the metastasis. Judged newly formed lymph vessels are present in this stroma. Clumps of cancer cells can be seen in these vessels and in a judged dilated preëxisting lymphatic of the tubal serosa and also in a lymph vessel (possibly a continuation of the preceding one) situated in the superficial portion of the muscularis (see Figs. 110 to 113 inclusive). If our observations and interpretations are correct the presence of newly formed lymph vessels in the stroma of this implant permit an earlier escape of the cancer into the lymphatic circulation than would occur if these vessels were not present. In addition, the expansion or actual outgrowth of the serosal lymph capillaries, similar to those shown in Case 6, can be seen in low granulation tissue about the margin of the implant. Every stage in the development of these dilated lymph vessels in this newly formed tissue is present (see Figs. 105 to 108 inclusive). These dilated lymphatics may play a very important rôle in the early escape of cancer cells into the lymphatic circulation from carcinoma implanted on the peritoneum. This may well occur even in very small implants under circumstances in which the portal of entry may easily be overlooked.

CASE 8. The patient, A. H. No. 92806, had carcinoma of both ovaries, associated with an extensive peritoneal carcinomatosis. Some of the findings in this case have been previously reported (see Case 4 of the former paper<sup>1</sup>). Photomicrographs of sections of two implants on the parietal peritoneum are shown in the present paper (see Figs. 118 to 120 inclusive). The conditions found in one of these implants possibly may represent the extension of carcinoma into a lymph vessel in the low granulation tissue on the surface of the peritoneum about the carcinoma.

## DISCUSSION

The material for this paper was obtained from eight patients with peritoneal carcinomatosis of ovarian origin. It was selected because it demonstrates the response of the lymphatics of the peritoneum to cancer cells which escape into the peritoneal cavity and become implanted on its serous surface. The origin of newly formed lymph vessels in the stroma of peritoneal implants is shown. The significance of these lymph vessels in the secondary spread of carcinoma is also indicated.



The various serosal reactions caused by cancer cells which escape into the peritoneal cavity from ovarian carcinomata have been described by the writer in a previous paper.<sup>1</sup> The most striking of these reactions is the development of patches of granulation tissue. These patches are most abundant in patients with early peritoneal carcinomatosis but they may be found at other times in the course of the disease. The granulation tissue assumes various structural forms depending on the manner in which the new tissue grows from the peritoneum and on the stage of its development. This new tissue forms the stroma of all polypoid implants as well as that of many of the other types mentioned in the first part of the present paper. Early in the development of peritoneal implants cancer cells become enmeshed in this tissue, but they are not necessarily found in all portions of it.

The polypoid implants are the ones that are best suited for the observation of possible lymph vessels in their stroma since all of the tissue which juts above the surface of the peritoneum must be newly formed and can readily be differentiated from the preëxisting tissues of the host. Studies are made of cancer-free polypoid granulation tissue first because, in the absence of the growing tumor cells, one may study this tissue more readily and so obtain all the information possible of its constituents. When carcinoma is present in this tissue the picture often becomes more complex, thus making it difficult to recognize all of the details. Further, one may reasonably conclude that the newly formed cancer-free peritoneal outgrowths caused by carcinoma consist of the same tissue as the stroma of true implants and that a careful study of the former will thus lead to a better understanding of the stroma of the latter. In this tissue vessel-like structures or spaces are found which usually accompany newly formed blood vessels. These spaces are lined by endothelium-like cells and appear to be continuous when followed in serial sections. Although their lumina are often empty, sometimes a few lymphocytes may be present within them. Frequently they may be followed from the place of attachment of the new tissue on the serosal surface to the very periphery of this tissue. These newly formed vessels in some instances can be traced to preëxisting lymph vessels in the underlying peritoneum, which are situated in the same stratum as are the blood vessels from which the newly formed blood vessels in the granulation tissue take origin. Newly formed lymph vessels in

granulation tissue may easily be confused with spaces created by the incomplete fusion of different portions of this tissue. When, however, their continuity with preëxisting lymphatics in the underlying peritoneum can be established their identity is assured. Unfortunately this continuity cannot always be detected because the underlying lymphatics may not be evident.

The fact that newly formed lymphatics cannot always be found in this granulation tissue is probably to be explained in one of two ways: either the inability to recognize non-injected lymphatics, even if they are present, when their walls are compressed and their lumina obliterated by pressure from the surrounding tissue, or the possibility that they are not always present. If the lymph capillaries in the organ or structure beneath the granulation tissue are more deeply situated than the blood capillaries, as is reported frequently to happen, they may be out of range of the stimulant causing the granulation tissue. Under these circumstances the newly formed blood vessels in this tissue may not be accompanied by newly formed lymph vessels. As might be expected, newly formed lymph vessels accompanying newly formed blood vessels are also found in granulation tissue in which cancer cells are embedded.

Preëxisting lymph vessels accompanying preëxisting blood vessels can be seen sometimes in the tissues beneath the attachment of the pedicles of mature polypoid implants to the peritoneum. Newly formed blood vessels accompanied by judged newly formed lymph vessels are found in the pedicles of these implants and also in their stroma above the pedicle. In one instance (Figs. 27 and 28) they are present in the stroma of the cortex of the implant, opposite its hilum, and can be traced backwards to very near the center of the implant where they are lost in the denser stroma in this situation. It is impossible to establish the continuity of the non-injected lymph vessels in the stroma of mature implants when they are subjected not only to the pressure exerted by the increased density of the tissues of the stroma but also to that of the growing carcinoma about them. The latter not only compresses these vessels but may also invade and destroy them. The evidence just presented indicates that the stroma of some of the polypoid implants contains lymph vessels and that these accompany or follow the blood vessels during the development of the granulation tissue stage of the implant. Judged newly formed lymph vessels are also found in non-polypoid encap-

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granulation tissue may easily be confused with spaces created by the incomplete fusion of different portions of this tissue. When, however, their continuity with preëxisting lymphatics in the underlying peritoneum can be established their identity is assured. Unfortunately this continuity cannot always be detected because the underlying lymphatics may not be evident.

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Preëxisting lymph vessels accompanying preëxisting blood vessels can be seen sometimes in the tissues beneath the attachment of the pedicles of mature polypoid implants to the peritoneum. Newly formed blood vessels accompanied by judged newly formed lymph vessels are found in the pedicles of these implants and also in their stroma above the pedicle. In one instance (Figs. 27 and 28) they are present in the stroma of the cortex of the implant, opposite its hilum, and can be traced backwards to very near the center of the implant where they are lost in the denser stroma in this situation. It is impossible to establish the continuity of the non-injected lymph vessels in the stroma of mature implants when they are subjected not only to the pressure exerted by the increased density of the tissues of the stroma but also to that of the growing carcinoma about them. The latter not only compresses these vessels but may also invade and destroy them. The evidence just presented indicates that the stroma of some of the polypoid implants contains lymph vessels and that these accompany or follow the blood vessels during the development of the granulation tissue stage of the implant. Judged newly formed lymph vessels are also found in non-polypoid encap-

sulations of carcinoma on the surface of the peritoneum (see Figs. 33, 34 and 35). These observations suggest that newly formed lymph vessels must also occur sometimes in the stroma of other malignant neoplasms containing newly formed blood vessels.

In one instance (Case 7) an early (granulation tissue stage) sessile polypoid implant is present on the surface of the Fallopian tube (see Fig. 102). This implant contains judged newly formed lymph vessels accompanying the newly formed blood vessels. Carcinoma is present not only in these lymph vessels but also in a preëxisting lymph vessel in the muscularis of the tube beneath the base of the implant. This suggests that newly formed lymph vessels in even a very early implant may be invaded by the carcinoma and thus cause an earlier spread of the neoplasm into the lymphatic circulation than would occur if they were not present. Others may claim that the presence of carcinoma in a lymph vessel beneath the base of the implant is not only an indication that the growth in this vessel reached its present situation from the primary ovarian carcinoma through the lymphatics, but also that the implant on the surface of the tube arose by this route. It is true that carcinoma reaching the subperitoneal tissues in any way may extend through the peritoneum to its surface and there cause a serosal reaction or even escape into the peritoneal cavity. The conditions found in this specimen in no way suggest the latter phenomenon (see Figs. 109 to 113 inclusive).

In some of the mature pedunculated implants carcinoma is found extending from the implants through their pedicles just as a primary carcinoma spreads. This extension may well have been through lymph vessels which have been demonstrated in the pedicles of similar implants not invaded by the carcinoma. In other implants the carcinoma not only extends through the pedicles but also is present in preëxisting lymph vessels beneath the attachments of the pedicles to the peritoneum. I believe that this latter condition may be a manifestation of the spread of carcinoma into the lymphatic circulation from the implant above it, in the cases which I have presented, rather than a retrograde lymph vessel permeation or metastasis from the original tumor (see Figs. 85 to 91 inclusive). Newly formed lymph vessels may well be present in the stroma of the implants just described but cannot be positively detected because of the scanty dense stroma present in these implants

and the compression and invasion of the vessels by the growing carcinoma.

A most interesting observation in this study is the presence of dilated lymph vessels in low granulation tissue which has replaced the peritoneum (see Figs. 97, 98 and 105 to 108 inclusive). Some of these vessels apparently are preëxisting and have expanded in this newly formed tissue. In other instances the tips of preëxisting vessels apparently grow outward and form blebs or even small cysts in the less dense granulation tissue. A similar tendency to bleb formation is observed in the terminal portions of lymph vessels reaching the tips of the tufts of luxuriant granulation tissue. The tips are the youngest and most rapidly growing portions of the tufts and are therefore the least dense. If small clumps of cancer cells should become embedded in low granulation tissue of the peritoneum, as sometimes occurs, and if dilated lymph vessels should be present in this tissue near the carcinoma, the neoplasm may easily invade these dilated vessels and thus gain access to the deeper preëxisting lymph vessels from which those in the granulation tissue arose. Later, lymphatic permeation and the development of metastases may result from this invasion. The then insignificant embedded implants in the thickened peritoneum may easily be overlooked in the attempt to discover the portal of entry of carcinoma into the lymphatics of the organ or structure beneath them. Possibly some of the ovarian carcinomata secondary to a primary growth in the intestinal tract may arise in this manner.

The spread of carcinoma in the lymphatics of patients with peritoneal carcinomatosis is an interesting problem. If carcinoma is present in the lymph vessels of any organ or structure and if newly formed tissue containing newly formed lymph vessels is present on its serosal surface, the neoplasm in the preëxisting lymph vessels may readily gain access to the newly formed lymph channels. Under these circumstances conditions may arise that resemble some of those illustrated in this paper (see Figs. 125 to 131 inclusive).

#### SUMMARY AND CONCLUSIONS

Cancer cells that escape into the peritoneal cavity frequently cause reactions of its serosal lining leading to the implantation of some of these cells on the surface of the peritoneum. The most im-

portant of these reactions for the study of the problem presented in this paper is the formation of various types of granulation tissue in which the cancer cells become enmeshed. Cancer cells, however, are not necessarily found in all portions of this tissue. This granulation tissue provides the stroma for many peritoneal implants.

In all parts of this granulation tissue spaces or vessels are found which are either empty or contain a few lymphocytes. They are lined by endothelium-like cells, usually accompany newly formed blood vessels, appear to be continuous when followed in serial sections and, in some instances, can be shown to take origin from pre-existing lymphatics in the underlying peritoneum. In other instances, however, for reasons which have been given, these spaces cannot be detected or, if evident, their continuity with preëxisting lymphatics cannot be determined. They are found in all types of granulation tissue in patients with peritoneal carcinomatosis. They occur in the low as well as in the polypoid masses of this tissue but are more readily seen in the polypoid group.

Certainly all of these spaces which can be shown to grow out from preëxisting lymphatics of the serosa into the overlying granulation tissue must be newly formed lymph vessels and, with newly formed blood vessels, take their part in the histogenesis of the stroma of carcinomatous implants. Some of these lymph vessel-like structures which have no apparent continuity with preëxisting lymphatics may be spaces created by the incomplete fusion of different portions of the growing granulation tissue. However, I believe that many of these unidentified structures are newly formed lymph vessels which, for various reasons, cannot be followed for their entire course. Therefore, the response of the serosal lymph capillaries to cancer cells which escape into the peritoneal cavity is, at least in some instances, similar to that of the blood capillaries and manifests itself by the growth of newly formed lymph vessels into the resulting granulation tissue on the surface of the peritoneum. This granulation tissue later forms the stroma of the mature implant.

Newly formed lymph vessels are found in all portions of the stroma of all types of mature peritoneal implants in which newly formed blood vessels are present even though they cannot be detected in the stroma of all of these implants.

Conditions are encountered in some of the implants which indicate that newly formed lymph vessels in their stroma permit an

earlier spread of the neoplasm into the lymphatic circulation of the host than would occur if they were not present.

The presence of dilated lymph vessels in low granulation tissue of the serosa in which clumps of cancer cells may become embedded deserves special attention. Some of these vessels apparently are pre-existing and have expanded in the new formed tissue. In other instances the tips of preëxisting vessels apparently grow outward and form blebs or even small cysts in the granulation tissue. These dilated lymph vessels may furnish a ready portal of entry into the lymphatic circulation for the carcinoma embedded in the tissues about them.

NOTE: The efficiency of the laboratory work for this paper is, in large measure, due to the technical skill and the interest of Miss Helen Buchan. The coloring of the photomicrographs was done by Mrs. M. R. Marden. The photomicrographs were made by Mr. James A. Glenn. These I thank for their interest and coöperation.

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## DESCRIPTION OF PLATES

### PLATE 52

FIG. 1. Photomicrograph of a section of a portion of a small patch of granulation tissue on the parietal peritoneum from a patient with a primary carcinoma of the left ovary associated with an early peritoneal carcinomatosis (Case 1). Carcinoma, however, was not found in any of many sections from this particular situation. Various sized tufts of granulation tissue are present. Of these the largest, "a," is composed of several smaller tufts which are partially fused. For newly formed blood and lymph vessels in tufts "a" and "b," see Figs. 3 to 12 inclusive.  $\times 10$ .

FIG. 2. A portion of the peritoneum between the tufts of granulation tissue shown in Fig. 1. The peritoneum has been stimulated, as indicated by its injected blood vessels and increased cellular elements. A large blood vessel "b.v.," filled with blood, appears in the center of the field. A branch arising from it extends toward the surface of the peritoneum. Two spaces, "a" and "b," representing either two separate lymphatics or portions of the same vessel, are present on either side of the large blood vessel. A third vessel, "c," possibly a branch of lymph vessel "b," can be seen extending upward. Note that the size and shape of the oval flattened nuclei of the endothelial cells lining lymph vessels "a" and "b" vary with the angle at which they are cut or seen in the section.  $\times 130$ .

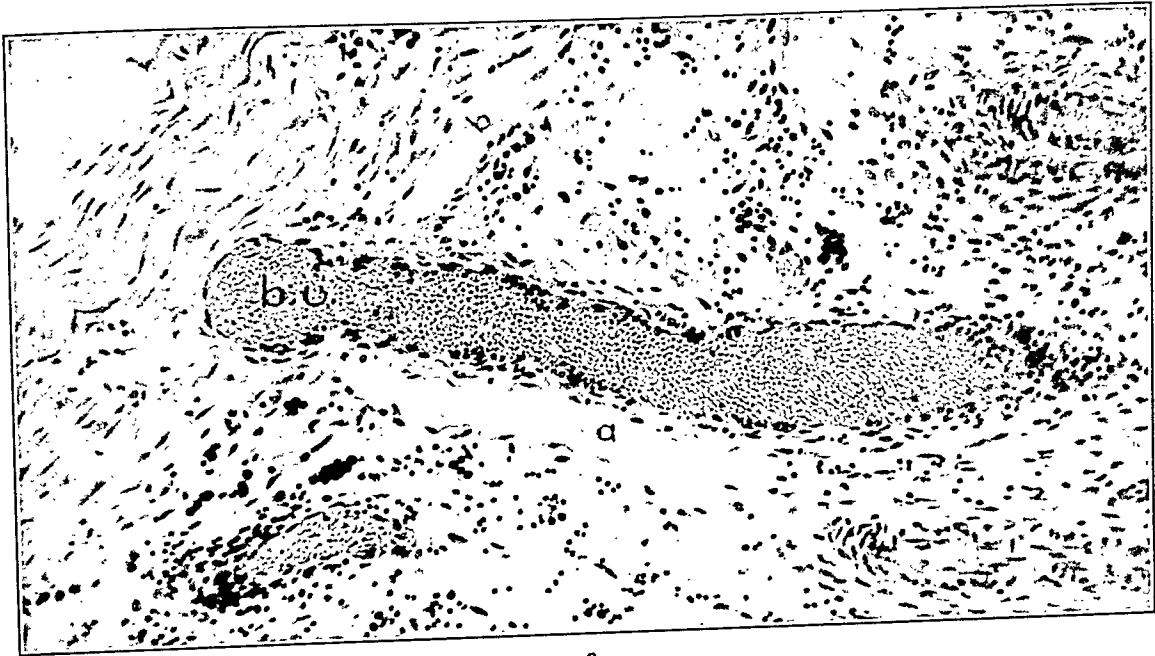
FIG. 3. A longitudinal section of a portion of the base of tuft "a" of the granulation tissue shown in Fig. 1. A cross-section of a blood vessel "b.v.," similar to the one in Fig. 2, is shown with a branch arising from it and extending into the granulation tissue (to the right in this photomicrograph). This branch is accompanied by a lymph vessel "a." The distal portions of both vessels are newly formed. For the farther extension of these vessels into the tuft of granulation tissue see Figs. 4, 5 and 6. A second lymph vessel or a branch of vessel "a" is indicated by "b." Its situation is similar to that of vessel "a" in Fig. 2. This photomicrograph indicates that the blood vessels are accompanied by lymph vessels in the formation of the granulation tissue.  $\times 130$ .



1



2



3

Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

PLATE 53

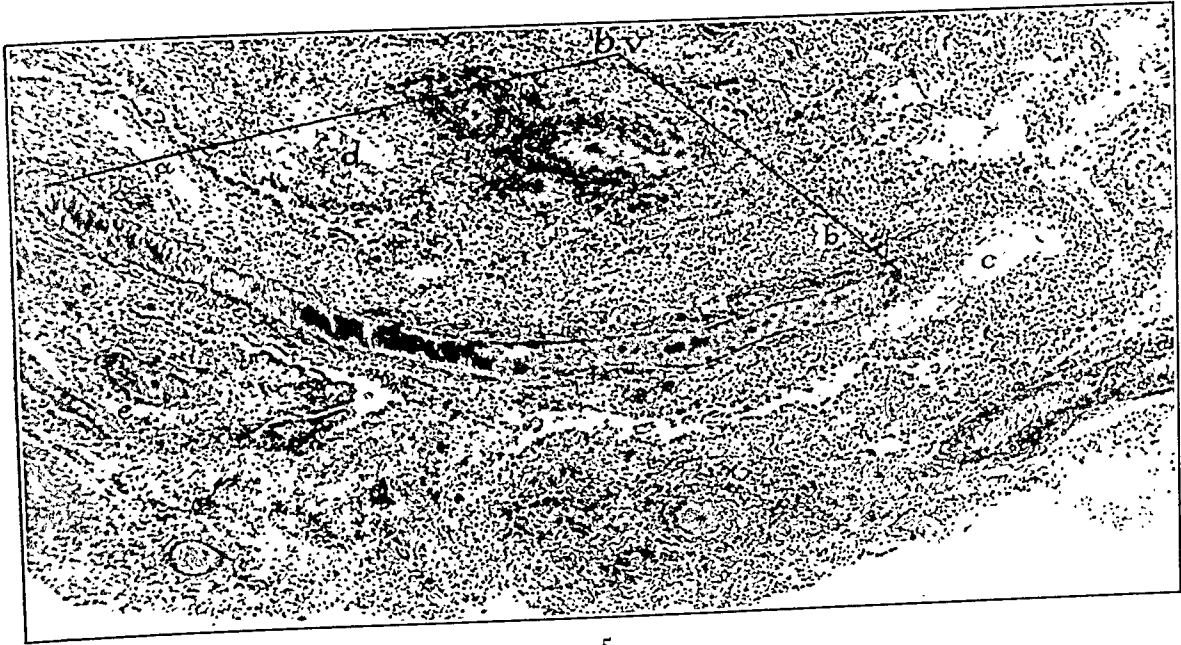
FIG. 4. Lower magnification of a section adjacent to that pictured in Fig. 3. All of the tissue in this photomicrograph is newly formed except a small portion of the peritoneum at the extreme left. The farther extension of both the newly formed blood vessel "b.v." and the newly formed lymph vessels "a" and "b" of Fig. 3 into the tuft of granulation tissue is shown. The blood vessel here divides into two large branches, as indicated by the arrows. Both of the branches accompanied by lymph vessels extend to the tips of different portions of the tuft of granulation tissue. Lymph vessels "b" and "c" accompany the upper of the two large branches, "e" the lower branch, and "d" another blood vessel.  $\times 54$ .

FIG. 5. Farther extension of the upper of the two large branches of blood vessel "b.v." of Fig. 4, accompanied by lymph vessels "b" and "c," into the tuft of granulation tissue is here shown. A longitudinal section of this branch is indicated by the pointer "b.v." Lymph vessels are shown at both ends of this portion of the blood vessel, see "a," "b" and "c." Similar lymphatics can be seen along the entire course of this blood vessel in other sections of this tuft. It is therefore believed that lymph vessels "a," "b" and "c" are continuous. A lymph vessel, "d," also accompanying blood vessels, is situated in an adjacent and partially fused tuft of granulation tissue. These newly formed structures can also be traced in other sections to pre-existing lymph and blood vessels in the peritoneum beneath the base of the tuft.  $\times 46$ .

FIG. 6. One of the tips of the tuft of granulation tissue indicated by "a" in Fig. 1. Blood vessels, branches of "b.v." of Fig. 5, are shown in cross-section to the right of the center of the photomicrograph. These are surrounded by dilated lymph channels which are continuous with those indicated by "b" and "c" of Fig. 5. The blood vessels may be followed from a pre-existing vessel of the peritoneum shown in Fig. 3, to the very tip of the granulation tissue (see also Fig. 8). The blood vessels, for their entire course, are accompanied by lymph vessels. The lymph vessel "a," which also accompanies blood vessels, is a continuation of the lymph vessel indicated by "d" in Fig. 5.  $\times 50$ .



4



5



6

FIG. 7. Higher magnification of the distal portion of the blood vessel "b.v." shown in Fig. 5. Lymph vessels "b" and "c," with lymphocytes in their lumina, are clearly seen on both sides of the blood vessel. Compare lymph vessel "c" with similar structures shown in Figs. 34 and 36.  $\times 130$ .

FIG. 8. Higher magnification of the tip of the tuft of granulation tissue shown in Fig. 6. The blood vessels are surrounded by lymph spaces, probably different portions of one lymph vessel. Both the blood and the lymph vessels are newly formed having developed from preëxisting vessels in the peritoneum from which the granulation tissue arises. It is this granulation tissue, produced in response to the stimulation of an ovarian carcinoma, yet containing no tumor cells, that must form the stroma of polypoid peritoneal implants. It is thus reasonable to assume that some of the implants would contain newly formed lymph as well as blood vessels if the latter are present in their stroma. Since the tip of the tuft of granulation tissue is the youngest and most actively growing portion its tissue is less dense than that in the older portions and its lymph vessels therefore are more dilated than those in the base of the tuft. Compare those pictured here with the lymph vessels shown in Fig. 3. Note the presence of lymphocytes in the lumina of these vessels and the apparent passage of these cells from the surrounding tissue through the endothelial lining of the vessels. It is not difficult to comprehend, then, that carcinoma growing in tissue containing lymph vessels like these may readily penetrate their thin endothelial walls, reach their lumina and, by continuous growth within these spaces, ultimately gain access to the preëxisting lymph vessels from which the newly formed vessels arise. Or, if continuous growth does not occur, emboli of cancer cells may reach the lymphatic circulation by the same route. It may be assumed, therefore, that carcinoma can spread more quickly from an implant containing newly formed lymph vessels than from one in which they are not present.  $\times 130$ .



7



8

Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants



PLATE 55

FIG. 9. Colored photomicrograph (higher magnification) of tuft "b" in the granulation tissue of Fig. 1. A lymph vessel, like the central lacteal of a villus of the small intestine, may be followed in this section from its origin "a" (see arrow) in a preëxisting vessel in the peritoneum, through the entire length of the tuft of granulation tissue to its tip. At "b" it is dilated, as is the lymph vessel in Fig. 8. Because it is slightly tortuous and in places constricted its entire length does not appear in the plane of this section. The dilated portion of the lymph vessel in the tip of this tuft resembles a small lake with a small stream arising from it, which flows into larger streams (the preëxisting lymph vessels of the peritoneum). Particulate matter, such as cancer cells, gaining access to the lumen of any portion of this newly formed lymph vessel might readily be carried downstream into the lymphatic circulation. A judged lymph vessel "c" is shown in an adjacent tuft of granulation tissue. The continuity of the channel "a"-"b" with a preëxisting lymph vessel in the peritoneum excludes its origin from the incomplete fusion of adjacent strands or tufts of granulation tissue during the early development of the latter (see Fig. 12). Other lymph vessels similar to this one are present in other sections of this tuft of granulation tissue.  $\times 54$ .

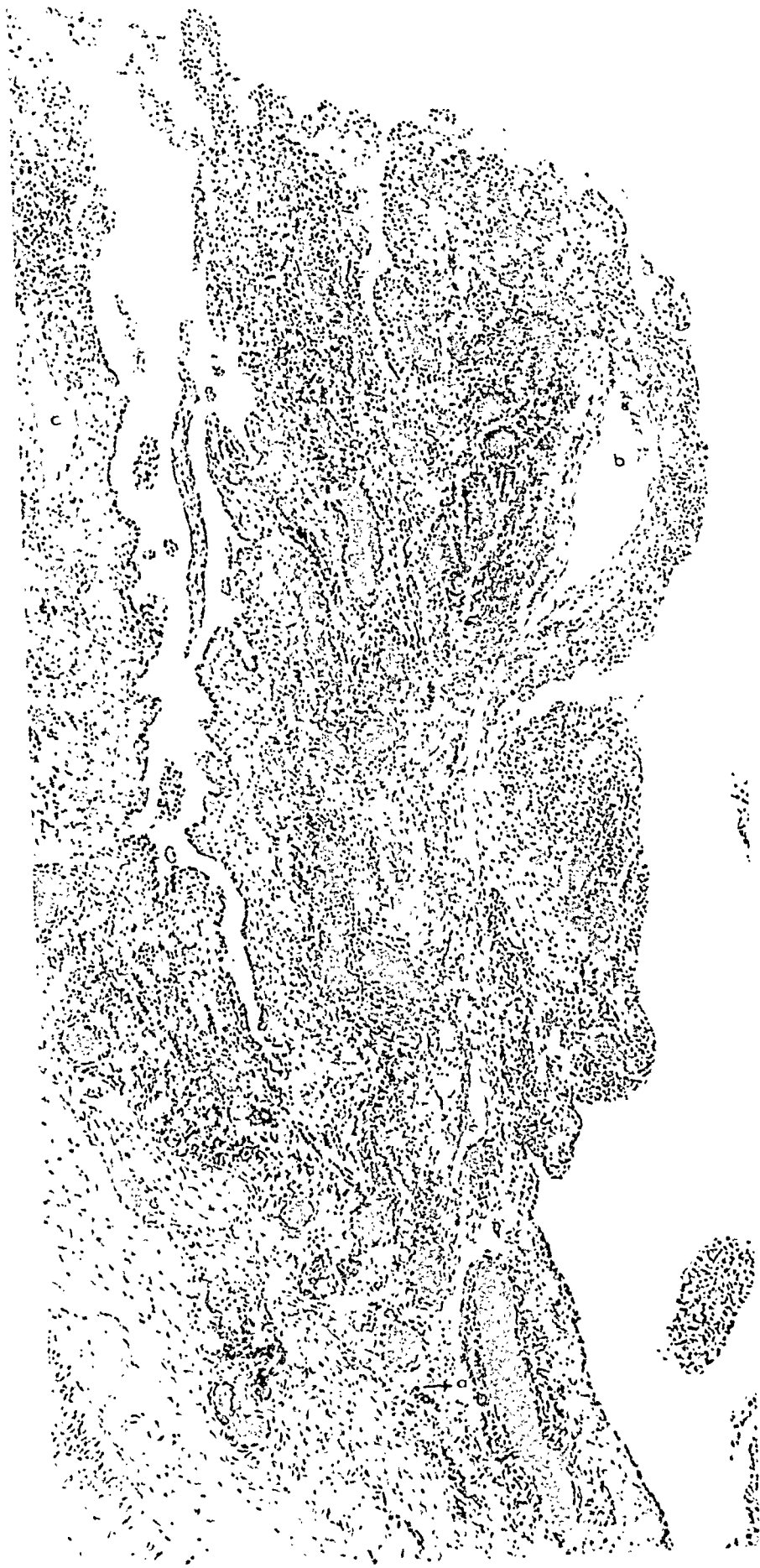


FIG. 10. Higher magnification of the dilated portion "b" of the newly formed lymph vessel in the tip of the tuft of granulation tissue shown in Fig. 9. This dilatation of the lymph vessel might be due to one of two factors. First if there were any interference with the outflow of lymph through narrow portions of the vessel in the base of the tuft a dilatation of that portion in the tip might easily occur since the tissue in this situation is relatively loose. Secondly, since Pullinger and Florey<sup>20</sup> have shown that lymph vessels, because of their intimate connection with the tissues surrounding them, become dilated in local edema the expansion of the growing tissue in this tip also may cause a local dilatation of the lymph vessel in it.  $\times 130$ .

FIG. 11. Higher magnification of the lymph vessel shown in Fig. 9, just below the portion shown in Fig. 10. The apparent variations in size of the lumen "a" and "b" and its absence "c," are due either to the undulating course of the vessel or to the normal fluctuations in the size of lymph vessels.  $\times 130$ .

FIG. 12. A portion of the base of tuft "b" in Fig. 1, also indicated by "a" in Fig. 9. A preëxisting blood vessel of the peritoneum accompanied by a preëxisting lymph vessel "a," is shown extending into the tuft of granulation tissue (to the left in this photomicrograph). The lumen of the lymph vessel appears to be nearly occluded at "b." As in "a," "b" and "c" of Fig. 9, this may result from the fact that the entire course of the lymph vessel is not in the plane of the section. It is very evident that it would be impossible to detect the presence of this lymph vessel if its lumen were occluded by pressure from the surrounding tissues because the nuclei of its endothelial lining would then be indistinguishable from those of nearby fibroblasts.  $\times 130$ .

isolated from the nasopharynx and spinal fluid during life, and from the brain and spinal cord of persons who have succumbed to encephalitis or poliomyelitis; it has also been done with streptococci from "natural" passage viruses and from milk and water supplies during epidemics of these diseases, and with certain strains of streptococci from sources remote from encephalitis or poliomyelitis. Neurotropic cataphoretic velocity and virulence of the streptococci appear to be essential for the production of the "virus" phase.

The symptoms and lesions in animals, especially monkeys, while variable at first, became more typical with successive passage. In the early "virus takes" the streptococcus could be re-isolated quite readily, but this became increasingly difficult with successive passages through animals; the streptococcus appeared and disappeared in successive cycles of from 3 to 5 or more passages. *Macacus rhesus* monkeys which had recovered with residual paralysis following inoculation of "natural" poliomyelitic virus proved resistant to highly virulent, experimentally produced virus. "Takes" at crucial points occurred simultaneously in more than one of a small series of animals inoculated and in two or three different species. Inoculated control animals never yielded transmissible virus or revealed lesions of encephalitis or poliomyelitis.

**ANTIBODY IN RELATION TO IMMUNITY TO ACUTE POLIOMYELITIS.** John A. Kolmer, with the assistance of Anna M. Rule, Philadelphia, Pa.

*Abstract.* The antiviral substance occurring in the blood appears to be a true antibody capable of neutralizing but not necessarily destroying the virus of poliomyelitis.

This antibody occurring in the blood of normal human beings appears to be identical in its properties with that produced by infection and vaccination of monkeys and human beings.

The origin of the natural antibody is uncertain but it may be the result of unrecognized infection, antigenic stimulation by common substances or happens occurring in various bacteria and other substances, or the product of normal growth.

The prophylactic value of this antibody in normal and immune serums for human beings is uncertain but probably slight and of short duration, due in part to inadequate dosage.

There is no irrefutable statistical proof of the efficacy of the antibody in the treatment of poliomyelitis of human beings nor any absolute proof of its complete ineffectiveness. It is possible and probable, however, that large amounts administered *early* in the disease may effectually prevent progressive infection of the spinal cord by the neutralization of virus.

The antibody, however, has demonstrated some protective activity in monkeys inoculated intracerebrally with virus and especially when administered in large amounts per body weight early in the incubation period.

The immunity produced in monkeys and human beings by infection or immunization with living virus (active, glycerinated and ricinoleated) appears to be largely cellular (tissue) but may be partly humoral due to antibody.

Over 90 per cent of monkeys immunized with living virus by various investigators showed the presence of antibody in the blood on the basis of serum neutralization tests. Practically 100 per cent injected subcutaneously or intracutaneously with 5 doses of ricinoleated virus in amounts of 0.1 to 0.5 cc. per kg. showed the presence of the antibody in the blood. In our laboratory the majority

of these monkeys were completely protected against the intracerebral injection of virulent virus and we believe that the resistance, while probably largely cellular, may be due in part at least to the antibody in the blood.

PHYSIOLOGICAL OR STRUCTURAL HOST FACTORS IN THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM INFLUENCING THE INVASIVENESS OF CERTAIN NEUROTROPIC VIRUSES AND THEIR POSSIBLE RÔLE IN THE EPIDEMIOLOGY OF POLIOMYELITIS. Albert B. Sabin (by invitation) and Peter K. Olitsky, New York City.

*Abstract.* Since the *rhesus* monkey, as was recently emphasized by Flexner, apparently does not respond to the virus of poliomyelitis in precisely the same manner as man, an attempt was made to discover, among other experimental virus diseases affecting chiefly the nervous system, a "model" for some of the unexplained facts in the epidemiology of poliomyelitis.

Studies on the virus of vesicular stomatitis in mice and guinea pigs have led to the conception that the progression of a neurotropic virus along the nerves from the periphery to the central nervous system (CNS) may be arrested at various sites as a result of certain physiological or structural host factors. These host factors vary with age and with the species of animal infected. Thus in young mice, virus introduced in the nose progresses along the olfactory nerve to the olfactory region of the brain, and from there to the thalamus, cortex, cerebellum, midbrain, and so on, giving rise to a fatal encephalomyelitis, while in older mice the virus reaches the olfactory region of the brain by the same route but stops there, not because of the insusceptibility of the remainder of the CNS but as a result of some local block; the older animals remain well and do not show any clinical signs of disease (see *Am. J. Path.*, 1935, 11, 839). In this instance, the block is central and develops with age in all but a few animals. When the virus is inoculated by other peripheral routes in mice there is also a block which develops with age but it appears to be peripheral. For example, after injection of the virus into the leg muscles of young mice it ascends along the sciatic nerve, enters the spinal cord, and thence upward to the rest of the CNS, giving rise to paralysis and ultimately death, while in older mice it enters neither the nerve nor the CNS and the animals remain well. Since inoculation of the virus directly into the sciatic nerve of older mice results in its further progression and development of paralysis and death as in the young, it appears that here the barrier is chiefly in some structure between the nerve and the muscle. After intraocular inoculation the virus is found to invade by way of the optic and fifth nerves in young mice, and again its invasion is arrested peripherally rather than centrally in older animals.

Variations of behavior of neurotropic viruses in different species may be considered on the basis of similar local blocks in the peripheral and central nervous system. While the CNS of young guinea pigs and mice is equally susceptible to the virus, peripheral inoculation rarely induces encephalomyelitis in the former, but does so uniformly in the young mice. Virus introduced in the nose of young guinea pigs appears to be arrested at the thalamic region and does not reach the cerebellum, midbrain, medulla and spinal cord; in older guinea pigs, however, the block is peripheral, for the virus does not reach even the olfactory bulbs. After injection into the leg muscles, the block is again peripheral, *i.e.* somewhere between the muscle and nerve, in both young and old guinea pigs, for inoculation

directly into the sciatic nerve results in the spread of the virus to the spinal cord and the remainder of the CNS, inducing paralysis and ultimately death.

Mice and guinea pigs, in which invasion of virus is sufficiently arrested to prevent clinical manifestations of disease, nevertheless develop specific antiviral bodies.

Certain analogies are clearly discernible between the manifestations of the experimental disease just described and certain phenomena encountered in the epidemiology of poliomyelitis. The possible rôle of structural or physiological host factors in the peripheral and central nervous system merits consideration as a hypothesis for the explanation of these phenomena.

*Discussion of papers by Drs. Kolmer, Sabin and Olitsky*

(Dr. Stuart Mudd, Philadelphia.) I presume there was no possibility that the adult animals could have had previous contact with the virus?

(Dr. Albert Sabin, New York City.) No. The possibility of spontaneous infection with this virus was excluded by the fact that the mice were carefully bred and kept under isolated conditions until used, that all were equally susceptible to infection by direct intracerebral inoculation, and by the demonstration that resistant animals contained no antibodies in their blood before exposure to the virus.

I should like to discuss Dr. Kolmer's paper. Dr. Kolmer's conclusions, if I understand them correctly, are (1) that the resistance of monkeys to poliomyelitis bears a definite relation to the content of antiviral body in their serum, (2) that the antibody content is greater in the serum of convalescent monkeys than in that of vaccinated monkeys, and (3) that the failure of Olitsky and Cox to induce an active resistance to infection with poliomyelitis in most monkeys treated with ricinoleated vaccine may be attributed to the fact that insufficient virus was administered. I regret to say that further studies on the same subject by Dr. Olitsky and myself have led us to adopt essentially opposite conclusions. Olitsky and Cox are not alone in maintaining that vaccinated monkeys may frequently exhibit appreciable antibody in the blood without being resistant to infection by way of the nose: Schultz and Gebhardt, Aycock, Hudson and co-workers have all obtained similar results using varying amounts of virus, both larger and smaller than that used by Dr. Kolmer. In a recent investigation, Dr. Olitsky and I undertook to determine in what way vaccinated and convalescent monkeys differed in resistance to infection by the nasal route and in the content of antibody in their blood. Convalescent monkeys were found to be resistant to reinfection with the same strain of virus when tested at intervals of 3 weeks to 3 months after the onset of paralysis. Antibody formation in them was very much slower, however. In none of the convalescent monkeys tested 1 month after onset of paralysis was there demonstrable antibody in the blood; some of them showed antibodies for the first time at 2 months, while others not until 3 months. We thus had convalescent monkeys without demonstrable antibody which were resistant to infection, and vaccinated monkeys with antibody which were not resistant. Titrations on the serums of convalescent monkeys which finally developed antibody indicated that quantitatively there was no appreciable difference between them and the serums of non-resistant vaccinated monkeys. Our conclusion for the present, therefore, is that the relation between antibodies in the blood and resistance of monkeys to infection with poliomyelitis is obscure, if

one actually exists, and that convalescents possess some mechanism of resistance which many vaccinated monkeys apparently lack.

(Dr. Kolmer, closing.) As stated in my paper, I believe it is very difficult to arrive at any definite conclusion on the rôle that antibody may play in resistance and immunity to poliomyelitis. But, while the protective or prophylactic activity of antibody in normal and immune serum has been of doubtful value in human beings, yet from the experiments of others and ourselves it would appear that large doses are capable of protecting a percentage of monkeys when given within a day or two after intracerebral or intranasal inoculation with virus.

Monkeys immunized with adequate amounts of vaccine carrying active virus almost always show the presence of antibody in the blood and the majority have proved resistant to intracerebral inoculation of virus. With my ricinoleated vaccine 5 doses of 0.1 cc. per kg. are not always sufficient and, unfortunately, investigators have not tested it for the presence of active virus by injecting 0.3 cc. intracerebrally in the monkey, as has been our custom. We never use a vaccine unless this preliminary test shows the presence of sufficient active virus to produce paralysis within 14 days after inoculation, as vaccines without this amount of virus have failed to immunize monkeys. We have not generally employed intranasal inoculation as a test for acquired immunity because of irregularity in infection of the controls. Whether or not the acquired immunity is due to humoral antibody or tissue resistance, I am not prepared to state. I believe, however, that active immunization of monkeys with my vaccine induces an important tissue resistance, but it is likely that the antiviral antibody in the blood may also play some part in the immunity.

#### A CLASSIFICATION OF PRIMARY INFLAMMATION OF ARTERIES. Howard T. Karsner, Cleveland, Ohio.

*Abstract.* The extension of inflammation so as to involve arteries is well known, as is also inflammation of arteries due to the direct invasion of parasitic agents. These inflammations are obviously secondary in character. There is a group of arterial inflammations which cannot be included in this category. In order to distinguish this group the term "primary" is suggested. In order to forward the study of these lesions a classification is proposed as follows:

##### ARTERITIS

- Acute* Alterative (degenerative)
  - Necrotizing
  - Ezudative
  - Vegetative (thrombo-arteritis)
  - Proliferative
  - Organizing
- Chronic* — combinations of
  - Intimal
  - Medial
  - Adventitial

The alterative or degenerative form of acute arteritis is observed in many infectious diseases, independently of direct bacterial invasion. Swelling and deterioration of muscularis and elastica are evident, often associated with edema, but without other signs of inflammation. That they are inflammatory is indi-

cated by the later appearance of definite exudation. The same is true of some of the necrotizing forms. The other terms are self explanatory.

Chronic arteritis differs from arteriosclerosis in distribution of fibrosis in the various coats and by delayed or absent secondary changes in the fibrous tissue.

### *Discussion*

(Dr. Paul Klemperer, New York City.) Dr. Karsner's classification is extremely interesting to me, and I think it will help to clarify the point of view of authors describing the histological lesions encountered. However, the difficulty, it seems to me, is not altogether removed in differentiating between the so-called degenerative and inflammatory lesions of arteries, particularly in chronic arterial lesions. I should like to ask Dr. Karsner how he would classify the lesions in the interlobular arteries in malignant nephrosclerosis. Does he consider them as inflammatory lesions, or closer to arteriosclerosis? This brings up again the old question as to whether one should regard arteriosclerosis as a degenerative or as a chronic inflammatory lesion.

(Dr. Alfred Plaut, New York City.) I should like to inject one point into this discussion of primary inflammatory arterial lesions, and that is the size of the arteries involved. One can see from Dr. Karsner's photomicrographs that in his material his attention was mostly directed to the smaller arteries, and there are occasional, and perhaps even frequent, instances where one sees inflammatory processes restricted to a certain size of artery or arteriole. In septic cases, if I may use that term, one sometimes finds precapillary arterioles, notably in the kidney pelvis and in the testicle, with necrotic lesions such as Dr. Karsner has shown. In very rare instances of arteritis in the lung, only vessels of a certain caliber are affected. In the vermiform appendix and occasionally in the ovaries, the precapillary arterioles sometimes are the seat of a chronic infectious process. We may, in future, have to make further subdivisions of the term artery, not only anatomically, but biologically, also. We probably will find that certain pathological processes, and especially certain immunological reactions, take place in arterial vessels of a definite caliber.

(Dr. Karsner, closing.) In reply to Dr. Klemperer, attention is drawn to the fact that this is a study of arteries rather than of arterioles. Technically, the examination of arteries is more readily made than is the case with arterioles. Thus in the study of the latter, matters of opinion are more likely to appear. I have the deepest respect for Dr. Klemperer's studies and opinion in regard to arteriolar disease. My own opinion is that the common forms of arteriolar disease, whether they be a malignant disease in young individuals, a superimposition of degenerative or exudative lesions on chronic arteriolar disease, or chronic disease itself, are forms of inflammation in the broad sense.

It is gratifying to have Dr. Plaut emphasize the curious manner in which disease of arteries is often limited to vessels within a certain range of size, may sometimes be generalized and may sometimes affect only one of a limited number of organs and regions. I am grateful for the criticisms of both Dr. Klemperer and Dr. Plaut.

(Dr. S. Burt Wolbach, Boston.) I deprecate the use of the words inflammatory and degenerative when specific connotations are implied in regard to lesions of unknown pathogenesis. Inflammatory reactions are often, possibly always, responses to extraneous factors brought into direct contact with tissues. The use of "degenerative," I think, should be employed to express changes in tissues



occasioned by factors which are injurious only through their effects on tissue metabolism.

Degenerative lesions, because of the mechanical weakness they occasion, may be followed by cellular and exudative responses commonly regarded as inflammatory. The histological sequences in experimental scurvy best illustrate this. I make a plea for great caution in the use of "degenerative" and "inflammatory" where etiological connotations are implied.

(Dr. Karsner, closing.) The nature of processes and conditions is of necessity subject to definition. Definitions are not mere collections of words but are orderly arrangements of words outlining or clarifying a concept. In the case of inflammation, the definitions, as is well known, vary greatly as to scope, and I choose to select the broadest of these definitions.

EXPERIMENTAL NEPHRITIS IN RATS INDUCED BY ANTIKIDNEY SERUM. Joseph E. Smadel (by invitation), New York City.

*Abstract.* Antiserums that are toxic for rats can be prepared in rabbits by injecting emulsions of perfused rat kidney. Such serums contain several factors; one of these affects the kidney primarily but is not strictly organ specific. This factor is absorbed by homologous kidney and liver cells and by fat-free kidney suspensions, but not by petrol-ether-soluble kidney lipoids, or by red blood cells or serum. It is found in the globulin fraction and occurs in varying amounts in some serums prepared by immunizing rabbits against other organs.

The nephrotoxic factor induces a disease characterized by albuminuria, cylindruria, anasarca, and elevated blood urea, without significant hematuria. The outstanding histopathological changes in the acute stage are tubular degeneration and thickening of the glomerular capillary basement membranes. Some rats recover to a greater or lesser degree. Chronic progressively affected animals, followed up to 10 months, show extensive tubular change—interstitial scarring, and thickened capillary basement membranes, crescent formation and glomerular scarring with various stages coexistent in the same area.

The proper amount of antikidney serum induced a less specific process characterized by the additional features of hematuria, death in 1 to 8 days and extensive fibrin thrombosis of glomerular capillaries. This apparently depends on the action of nephrotoxin in conjunction with other factors.

*Discussion*

(Dr. David P. Seecof, Montreal.) I should like to ask whether controls were available of animals kept under similar conditions, say for 200 days. In a colony of rats that have been inbred for a long time it is not rare to find such renal lesions. These animals never received nephrotoxin.

(Dr. James P. O'Hare, Boston.) Were there any acute changes outside the kidney comparable to those in the kidney? Chronic changes you showed, but I wonder if there were acute changes as well.

(Dr. Smadel, closing.) The acute phase of nephrotoxic nephritis can be induced without accompanying significant lesions in organs other than the kidney; the anaphylactoid reaction must be avoided, however. When the anaphylactic-like response is severe enough to result in hematuria and fibrin thrombi in the glomerular capillaries then other organs are affected, and a general vascular phenomenon with hemorrhage and transudation is observed.

Between 350 and 400 rats were used in these experiments: slightly over half received nephrotoxic serums; the remainder received other antisera or were uninoculated. Approximately 50 of this latter group were observed for from 6 months to a year; 20 of these, uninoculated controls, were kept on a diet of only bread and milk. Some of the animals on this poor diet developed albuminuria and hematuria and were found to have pyelonephritis; *B. enteritidis* was isolated from the kidney. Chronic lesions comparable to those developing after nephrotoxin were not observed in control animals.

THE INADEQUACY OF ALLERGIC INFLAMMATION IN PROTECTION AGAINST INFECTION WITH VIRULENT PNEUMOCOCCI. Paul R. Cannon and (by invitation) George Hartley, Jr., Chicago, Ill.

*Abstract.* These studies were undertaken in an attempt to determine to what extent allergic inflammation may modify infection with a virulent pneumococcus. Rabbits were made allergic by repeated subcutaneous injections of egg albumin until they gave positive Arthus reactions. Other animals were actively immunized by the subcutaneous injection of formalin-killed pneumococci. Later, these animals and normal controls were injected subcutaneously with graded dilutions of living Type I pneumococci suspended in a solution of egg albumin. If allergic inflammation occurs quickly and effectively, it should alter the ability of the pneumococci to escape from the site of infection, and if adequate, should protect the animals when comparatively few bacteria are injected.

The results showed no protection whatever. Allergic animals, when injected with only 50 living pneumococci, died as quickly as the normal controls. Even when the allergic inflammation was present for 2 to 3 hours before injection of the pneumococci into the site of inflammation, the animals died as promptly as did the controls. Immune animals, on the other hand, survived much larger doses of infecting organisms, demonstrating the superiority of immunity over allergic inflammation, when dealing with highly virulent microorganisms.

*Discussion*

(Dr. George Packer Berry, Rochester, N. Y.) I wish to report a situation with a filterable virus analogous to that just described by Dr. Cannon. For a number of years we have been studying the relation of the viruses of rabbit fibroma (Shope) and infectious myxomatosis (Sanarelli). Recently we described a method for changing fibroma virus, which produces a benign type of local infection, into myxoma virus, and which always leads to a generalized reaction which invariably kills domestic rabbits. In attempting transformation experiments in the reverse direction, *i.e.* from myxoma virus to fibroma virus, we have attempted many procedures designed to localize myxoma virus to the site of inoculation. With this objective we have tried to fix myxoma virus in areas of allergic inflammation, induced in different ways by a variety of suitable proteins. All our attempts failed. As in Dr. Cannon's experiments, allergic inflammation was inadequate in preventing generalization of the infectious agent.

(Dr. Esmond R. Long, Philadelphia.) In connection with the experiments which Dr. Cannon has reported, and also those to which Dr. Rich referred this morning, I wish to comment on an experiment which my colleague, Dr. Lurie, has performed. Dr. Rich pointed out that it has always seemed plausible that the overwhelming inflammation in the allergic reaction would be the factor re-

sponsible for the rapid destruction of bacteria. Contrary to the original experiments on the rapid localization of bacteria in allergic inflammation, Dr. Lurie had this experience. If he injected similar quantities of tubercle bacilli into the legs of normal and tuberculous rabbits and guinea pigs and counted the number of bacteria found 24 hours later in the regional lymph nodes, he discovered, contrary to what might be expected on the basis of rapid localization, that there were more tubercle bacilli in the animals that gave the allergic reaction than in the others. If the tubercle bacilli were mixed with Trypan blue or India ink before injection, the color of the lymph nodes gave similar evidence of more rapid drainage in the allergic animals. Subsequent to the acute response the bacilli were destroyed more rapidly in the allergic animal, both in the lymph nodes and at the site of injection. If he mixed tubercle bacilli with agar and injected them into animals, so that they were held mechanically, in those animals with allergic inflammation a rather rapid concentration and destruction of the tubercle bacilli took place, in spite of the fact that cells of inflammation could not reach many of them. This is in agreement with Dr. Cannon's experiments, and indicates that there is something outside of the cells, perhaps produced from them which is responsible for the retardation of growth of tubercle bacilli, and ultimately destroys them. This changes our conception of the way in which the allergic reaction is responsible for the localization of bacilli.

THE PHAGOCYtic ACTIVITY OF CIRCULATING CELLS IN LEUKEMIAS. Max M. Strumia, Bryn Mawr, Pa.

*Abstract.* Experiments on phagocytosis, employing leukocytes circulating in normal subjects as well as all forms of acute and chronic leukemias, and in the so-called glandular fever (acute mononucleosis) gave the following results:

*Normal Blood:* Maximal phagocytic activity is displayed by polymorphonuclear neutrophils followed closely by monocytes. Eosinophils also have distinct phagocytic activity, but less than that of previous groups. Lymphocytic cells display no phagocytic activity whatever.

*Chronic Lymphatic Leukemia (1 Case) and Acute Lymphatic Leukemia (1 Case):* Undifferentiated oxidase-negative cells (hemocytoblasts), lymphoblasts, prolymphocytes, lymphocytes, and leukocytoid lymphocytes showed no phagocytosis. Neutrophilic cells in varying numbers showed normal phagocytic activity.

*Acute Myelogenous Leukemia (1 Case), and Chronic Myelogenous Leukemia (2 Cases):* Hemocytoblastic undifferentiated oxidase-positive cells show doubtful or no phagocytosis, myeloblasts very slight to doubtful phagocytosis, and promyelocytes very slight phagocytosis. A sharp increase in the phagocytic activity occurs with the myelocytes and increases to a maximum with the neutrophilic polymorphonuclears. This appears to be coincidental with the development of the mature specific neutrophilic granulations. There is practically no difference in the phagocytic ability between the mature neutrophilic polymorphonuclear and the young neutrophils (rod-nuclears and metamyelocytes) if one takes into consideration the relative amount of cytoplasm.

*Acute Hemohistioblastic (Monoblastic) Leukemia (2 Cases):* The large undifferentiated hemohistioblasts (reticuloendothelial cells) show active phagocytosis, in sharp contradistinction with the undifferentiated cells occurring in acute myelogenous and acute lymphatic leukemias, which show no phagocytosis.

Monoblasts show slightly less phagocytosis. Monocytes show phagocytosis as in normal blood.

*Glandular Fever (Acute Mononucleosis):* The typical cells erroneously interpreted as monocytes, actually young degenerated lymphocytic cells, show no phagocytosis.

#### Discussion

(Dr. Jacob Furth, New York City.) May I ask whether Dr. Strumia studied normal bone marrow and compared the phagocytic activity of immature normal bone marrow cells with that of leukemic cells? Leukemic cells are probably malignant cells, and their phagocytic activity cannot be taken as an indication of what normal cells would do.

(Dr. Paul R. Cannon, Chicago.) We have made similar observations in a casual way in a patient with myelogenous leukemia. We observed the same gradient of phagocytosis for staphylococci that Dr. Strumia has found in this systematic study. We found that the immature leukocytes were but slightly phagocytic and that the degree of phagocytosis was correlated directly with the degree of maturity of the leukocytes in the leukemic blood.

(Dr. L. D. Fothergill, Boston.) I should like to mention that Dr. Diamond and I made a similar study a couple of years ago, which was not published, but which entirely confirms Dr. Strumia's results. Our study differed only in minor details. We used a rough pneumococcus, for example, instead of a staphylococcus, but our results were identical with those reported by Dr. Strumia.

(Dr. Charles Weiss, San Francisco.) May I ask Dr. Strumia whether any attempt was made to determine the relative bactericidal power of the various types of leukocytes?

(Dr. Strumia, closing.) We did quite a number of experiments with bone marrow emulsions, and also with a variety of exudative cells. The most striking one was from a sternum biopsy of a case of hemohistioblastic leukemia, the patient dying about 3 hours later. The emulsion of bone marrow was almost entirely made up of very large cells of reticuloendothelial nature, and those cells picked up everything particulate that was present in the suspension; they phagocytosed red cells and cellular fragments of all sorts, carbon granules and some collodion particles that had been lying in the laboratory for a number of years, as well as all sorts of bacteria. The phagocytic activity of these cells seemed to be less strictly dependent on the concentration of blood serum than occurs with other types of cells. I cannot make numerical statements because of the difficulty of washing these suspensions free of serum without injuring the cells. We also used an emulsion of splenic pulp from a case of acute infection showing engorgement of the sinuses with all sorts of young granulocytic cells; also emulsions of normal bone marrow, and the results seem to correspond very closely to what we presented.

In response to Dr. Weiss' question, we have made no observations whatever of the lytic power of these various cells toward ingested organisms.

OBSERVATIONS CONCERNING THE TITRATION OF VIRULENCE. Robert M. Pike (by invitation) and G. M. MacKenzie, Cooperstown, N. Y.

*Abstract.* The importance of conclusions to be drawn from attempts to identify with virulence some constituent of the bacterial cell seems to warrant a careful examination of the variable factors involved in the determination of virulence.

In seeking to control these factors in the titration of the virulence of *Salmonella aertrycke* for white mice, we have listed a number of variables which may affect the accuracy of the results unless properly controlled or accounted for. These variables include the different criteria in use for measuring virulence, the variables concerning the inoculum, and those concerning the test animal.

In considering the available criteria for measuring virulence, we find that accurate determination of the *minimum lethal dose* of *Salmonella aertrycke* cultures requires too many animals to be practicable, as has been shown by Lockhart and by Trevan. An analysis of the results obtained in a series of virulence tests, employing 25 mice in each test, reveals that *percentage mortality* and *mean survival time* of test animals are in general parallel although there are instances where a false idea of virulence will be obtained unless both these criteria are considered. The *mean time to death* appears, in our experiments, to be a less accurate and less reliable measure of virulence, except in tests in which the mortality is close to or actually 100 per cent.

Following the observation that the death rate increases relatively slowly with increase in the dose, it has been maintained by Wilson and by Lockhart that minor variations in the size of the inoculum, such as those that result from inaccuracies in technic, have no significant effect on the result. With most cultures, especially those of intermediate and low virulence, we find, however, that there is a critical range of dosage in which doubling the dose may decrease the survival time as much as 30 per cent. Accurate standardization of the inoculum is therefore of utmost importance.

The danger of drawing conclusions in regard to virulence from the results obtained with very small numbers of animals is again emphasized.

PHAGOCYTOSIS OF HEMOLYTIC STREPTOCOCCI. Stuart Mudd and (by invitation) Horace Pettit, David Lackman and E. J. Czarnetzky, Philadelphia, Pa.

*Abstract.* Phagocytosis of hemolytic streptococci by washed exudative rabbit leukocytes in the presence of serial dilutions of serums has afforded a sensitive analytical method. The phagocytosis-promoting effects of serums in general have paralleled their mouse protective values. Phagocytosis and protective effects are type-specific for Griffith types, but with some minor cross reaction.

The "C" substance as prepared by Lancefield's method has been found to contain a certain proportion of another antigen which gives a precipitate with any antiserum prepared against the *Beta*-hemolytic streptococci. This "C" substance which has been further purified (C' substance) has given precipitates with serums only against certain types of Group A. Absorptions of Griffith Type I antisera with the fractions of Lancefield's "C" substance, and with "M" substance from a homologous strain fail to reduce their phagocytosis-promoting value; indeed such absorptions increase phagocytosis, possibly because of removal of inhibiting serum lipoids.

A heavy suspension of hemolytic streptococci may be thoroughly disintegrated by exposure for 1 hour to intense sonic vibration produced by a magnetostriction oscillator of design described by Chambers and Gaines (*J. Cell. & Comp. Physiol.*, 1932, 1, 451), and Chambers and Flosdorf (*Proc. Soc. Exper. Biol. & Med.*, in press). When the bacterial debris has been thrown down by centrifugalization, the decanted supernatant fluid is found to contain a solute which will completely absorb the phagocytosis-promoting antibody from homologous rabbit antiserum.<sup>1</sup>

Acidification with hydrochloric acid to certain characteristic pH values causes precipitation of a fraction (designated P) from the supernatant fluid from sonic disintegration of streptococci. This fraction has the property of precipitating the phagocytosis-promoting antibody from the specific antiserum. The neutralized fluid remaining after such acid precipitation is inactive against the phagocytosis-promoting antibody. The supernatant fluid from the sonic disintegration may be passed through a Berkefeld filter and preserved by the lyophile process without losing its activity.

This newly discovered component is exceedingly labile. It has been deprived of most or all of its antiphagocytic activity by: (a) heating to 56° C. for 30 minutes; (b) keeping in 1 per cent formaldehyde at neutral reaction for 10 minutes; and (c) keeping at pH 10.0 for 10 minutes.

An acid reaction, pH 2.0, for 10 minutes only slightly reduced the activity of the labile substance. Experiments are under way to test the capacity of this labile bacterial component to elicit active immunity in mice and rabbits.

CHEMOTROPISM OF LEUKOCYTES: THE SOURCE OF SUBSTANCES ATTRACTING MONOCYTES TO BACTERIA. Harold M. Dixon (by invitation) and Morton McCutcheon, Philadelphia, Pa.

*Abstract.* Earlier observations showed that human polymorphonuclear leukocytes, as observed with the microscope *in vitro*, were strongly attracted by all of a number of diverse types of microorganisms, with nearly equal intensity, as computed from the direction of the leukocyte's paths. The nearly uniform reaction of leukocytes to different microorganisms suggested a chemotropic factor common to all. This factor was apparently not the size of the bacteria, since carbon particles of approximately the same size as bacteria excited no reaction on the part of leukocytes. The culture medium was next investigated, since all the microorganisms were taken from artificial media, from which substances attractive for leukocytes might adhere to the bacteria. It seemed possible that such substances, if present, could be removed from the bacteria by repeatedly washing them with NaCl solution or distilled water, but this procedure failed to decrease their attraction for leukocytes. Further experiments were then made to decide whether the culture medium or the bacteria themselves were the source of the attraction. A drop of infusion broth dried on a glass slide failed to attract leukocytes. *B. coli* were then grown in broth, which was subsequently passed through a Berkefeld filter. A drop of the bacteria-free filtrate was found to attract leukocytes with moderate intensity. It was therefore concluded that it was the bacteria rather than the culture medium that attracted leukocytes. Experiments were then designed to answer the question whether, *in vitro*, bacteria directly attract leukocytes, or whether they do so indirectly through injuring leukocytes which themselves then give off attractive substances. It was found that when leukocytes were trapped on the margin of a mass of bacteria and became immobile, other leukocytes did not pile up on the trapped cells but arranged themselves at random on the edge of the bacterial clump, as would be expected if the bacteria attracted them directly. From these and other experiments it is concluded that under the present conditions chemotropism to bacteria is due to some substance or substances which are given off by the bacteria and which excite a directional response on the part of the leukocyte.

*Discussion*

(Dr. Virgil H. Moon, Philadelphia.) I am highly interested in the experiments related here, because they have a direct bearing on certain phenomena associated with inflammation, particularly that due to bacterial or infectious agents. In experiments on inflammation not incited by bacteria, but resulting from a sterile burn of the skin, we noticed that leukocytes were drawn in large numbers and very early to the area of injury. In this experiment no bacteria of any kind were concerned. The results indicated that some substance derived from the injured cells attracted bacteria to the area of injury. I should like to know whether Drs. Dixon and McCutcheon have tried the chemotropic effect of injured tissue, as epidermal cells or other injured cells, for leukocytes.

(Dr. Dixon.) In answer to Dr. Moon's question, leukocytes were taken from the buffy coat of human blood, dried, and then tested for chemotropic substances. We found that other leukocytes were attracted very weakly by the dried cells.

(Dr. Moon.) This would indicate that the leukocytes themselves do not give forth a chemotropic substance, but it does not indicate that the other cells of the body when influenced by injury, may not give forth a chemotropic substance. It seems to me that similar experiments should be tried on tissue cells.

SERUM ESTERASE IN RATS INJECTED INTRAPERITONEALLY WITH FRESH MACERATED TISSUES.\* F. A. McJunkin and (by invitation) G. A. Hemwall and E. A. Fullgrabe, Chicago, Ill.

*Abstract.* Although Shaw-Mackenzie (*Proc. Physiol. Soc., London*, 1911, 42, 11) in 1911 observed in cancer-resistant mice an increased capacity of the serum to activate lipase, Green (*Brit. J. Exper. Path.*, 1934, 15, 1) appears to have been the first to determine the ester-hydrolysing property of serum in mammalian malignancy. However, Falk (*The Chemistry of Enzyme Actions*, 1934, Ed. 2, N. Y.) had studied the esterase content of a number of normal and malignant tissues and states: "The first striking fact which may be mentioned is the small value of the carcinoma (Flexner-Jobling) on all esters." Green found a definite fall in the esterase content of the serum even at a very early stage of the tumor (Jensen sarcoma) growth. Sure, Kik and Buchanan (*Biochem. J.*, 1935, 29, 1508) working with Walker rat tumor No. 256 have confirmed the findings of Green. Directly following Green's publication 2 years ago we made esterase determinations on a number of rats inoculated with a spindle cell sarcoma which was primary in the uterus and which yields 90 per cent of positive "takes" when inoculated by the pocket method. The Green

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\* Since the above report was made additional results have been obtained. Cholesterol has been found to reduce esterase to a much greater extent than any of the other constituents of the tumor which were tested. The esterase in 22 rats which received an average dose of 133 mg. of cholesterol was reduced to 13.5, a decrease of 9.6 or 37.9 per cent after allowing for the "bleeding effect." Since such small doses of cholesterol, entirely without toxic effects so far as could be determined, reduce the esterase even more than 2 gm. of fresh tumor tissue, it seems to us that the cholesterol content of tissues may play a prominent rôle in the reduction of esterase that follows macerated tissue injections, and that the reduced esterase activity of sarcoma-bearing rats may be explained by absorption of cholesterol from the tumors.

technic was followed with the notable exception that the samples of blood were withdrawn from the heart of the living rat without the use of anesthetic. In 211 normal rats (weight 150 to 275 gm.) the serum had an average esterase content of 25.3 (cc. N/100 NaOH to neutralize fatty acid liberated from ethyl butyrate). In 20 sarcoma-bearing rats (average age of tumor 28 days) the esterase was 13.5, a decrease of 11.8 (46.6 per cent) below the normal mean. We then sought an explanation for the esterase reduction by injecting constituents of the tumor and of rat tissues. Fats (olive oil), fatty acids (oleic acid), and phospholipids (lecithin (Eastman practical)) were injected. Serum esterase determinations were made 24 hours after the injections. Of these only lecithin in large doses (650 mg.) brought about an esterase reduction which amounted to only 3 after allowing 2.2 for the "bleeding effect." To determine the "bleeding effect" blood (2-3 cc.) was withdrawn from the heart of 52 normal rats and 24 hours later samples were again removed. The esterase average of the second samples, which represents the effect of a single bleeding, was 2.2 less than that of the initial ones. Attention was turned to the non-lipoid portions of the tissues and fresh macerated sarcoma was injected. Two gm. of the fresh macerated tumor injected intraperitoneally caused an esterase decrease of 32.4 per cent after allowing for "bleeding effect." Four gm. of fresh macerated rat liver resulted in a decrease of 24.9 per cent after allowing for "bleeding effect." In a small group of 8 rats large doses of Witte's peptone which approached the lethal dosage reduced the esterase 35.7 per cent after allowing for "bleeding effect." Smaller doses produced no regular effect. Injections of fresh macerated rat sarcoma result in esterase reductions comparable to the decreases observed in rats bearing large tumor growths. It is quite possible that absorption of protein split products from the autolyzing tissues and of phospholipids may account for the esterase decreases. However, it is felt that additional experiments are required to explain the results.

MYASTHENIA GRAVIS: AUTOPSY FINDINGS IN A CASE. Charles F. Branch, Boston, Mass.

*Abstract.* A case of myasthenia gravis in a white adult female 23 years of age is presented. The clinical course was characteristic. The autopsy findings were essentially similar to those reported by other observers with the following exceptions: No thymic tumor was present; on the contrary, a distinct atrophy of the thymus was noted. No central nervous system lesions were found. Numerous minute, acute inflammatory lesions were found in the striated and cardiac muscle, associated with the usual lymphocytic infiltration described by others. The author was unable to demonstrate the presence of streptococci in these lesions.

*Discussion*

(Dr. Edward C. Rosenow, Rochester, Minn.) In Dr. Butt's work, when he stained his sections by the usual Gram method, he found no organisms. When he stained them in like manner, but only partially decolorized them, a method which we have used successfully many times in other studies, he obtained diplococci in all of his cases. We have studied 49 patients having myasthenia gravis and we have found consistently a streptococcus in the nasopharynx, tonsils, infected teeth, urine, and excised muscle which, when injected into monkeys and rabbits, localized selectively in muscles. If large doses were given, there was a



primary reaction in which weakness was a pronounced symptom, following which apparent recovery ensued, which in turn was followed long after injection by progressive weakness and fatigability, and finally great loss of weight. Cultures taken from the muscles after death have yielded streptococci with great regularity, whereas cultures from the blood and other viscera were nearly always sterile. Sections of the muscles of monkeys and rabbits which developed symptoms resembling myasthenia gravis revealed lymphocytic collections and other lesions resembling those of myasthenia gravis.

(Dr. Branch, closing.) In staining our slides we used the method suggested by Dr. Butt.

**BLASTOMYCOSIS OF THE HEART.** R. D. Baker and E. W. Brian (by invitation), Durham, N. C.

*Abstract.* Blastomycosis of the heart was encountered at autopsy in 2 cases of generalized infection with *Blastomyces dermatitidis*. Each showed diffuse pericardial blastomycosis, a large blastomycotic tubercle of the right atrial wall, and involvement of the corresponding endocardium. From the latter site organisms apparently entered the blood stream to produce the miliary pulmonary blastomycosis noted in both cases. Evidences of cardiac insufficiency, dependent probably on the cardiac blastomycosis, occurred in both.

Blastomycosis of the heart may also develop as part of a generalized miliary blastomycosis, and possibly by retrograde lymphatic extension from the infection in mediastinal nodes.

Blastomycosis is similar to tuberculosis in respect to cardiac involvement.

**EMBOLIC PULMONARY LESIONS PRODUCED IN RABBITS BY HUMAN FAT CONTAINING FATTY ACIDS OR SOAPS (CA. SR. BA.).** Edwin F. Hirsch, Chicago, Ill.

*Abstract.* The fatty acids of oil systems in contact with alkaline aqueous solutions exchange H-ions for the basic ions dissolved in the aqueous fluid.

Fatty acids in such oil systems are solvents for certain soaps insoluble in aqueous solutions.

Human fat containing fatty acids abstracts base ions from contacting aqueous liquids and the soaps so formed are important in determining the subsequent tissue reactions.

The exchange of H-ions and base-ions at the oil-water interphase when an oil contains dissolved fatty acid is an important link between the chemical reactions of the two immiscible systems.

Human fat with a comparatively high content of oleic or stearic acid produced in the lungs of rabbits a marked fibroblastic tissue reaction, the stearic acid mixture some large foreign body giant cells.

Human fat containing smaller concentrations of oleic and stearic acids neutralized with calcium hydroxide stimulated leukocytic exudates and a moderate fibroblastic tissue response. After neutralization with strontium or barium hydroxide it stimulated a marked fibroblastic tissue, epithelioid and giant cell reaction.

The complete report will be published in the *Archives of Pathology*.

## POSTOPERATIVE PULMONARY EMBOLISM. J. S. McCartney, Minneapolis, Minn.

*Abstract.* In a series of 12,500 consecutive autopsies, death in 2058 instances was classified as postoperative. Of the deaths so classified, 1303 were in males and 756 in females, a ratio of 1.7 to 1. The number of fatal pulmonary embolisms in males was 64 and in females 41 or percentage incidences of 4.9 and 5.4 respectively. When adults only were considered, these percentages became 5.6 and 5.7.

Examination of the records according to the site of operation showed no significant difference between abdominal and extra-abdominal operations as forerunners of pulmonary embolism. Eighty-three embolisms followed abdominal operations and 22 followed extra-abdominal operative procedures. In the group of abdominal operations there was a marked difference in the number of embolisms following surgical procedures on the various organs. Operations on the prostate and bladder led with 18 emboli, herniotomies accounted for 14 and operations on the uterus and adnexae for 12. Herniotomy showed the highest percentage, 19.4; the prostate and bladder next, with 11.7; and the incidence was 10.3 following abdominal operations on the uterus and adnexae. It was of interest that the incidence following herniotomy was 24 in males and 9.1 in females. The incidence following appendectomy was 6 per cent. Sixty-six operations on the perineum and external genitals caused 7 fatal embolisms.

Comparison of the age distribution of the cases showed almost identical percentages for the two sexes in the different decades. However, a similar comparison of the age distributions of the embolisms showed apparent sex differences, the embolisms appearing at an earlier age in females but lasting to a later age in males. A possible explanation for this was the fact that operations on the uterus and adnexa took place for the most part much earlier in life than did operations on the prostate and bladder. Herniotomies occupied a position between these two sites.

*Discussion*

(Dr. Edward C. Rosenow, Rochester, Minn.) I should like to ask whether there was any seasonal incidence in the number of postoperative deaths from pulmonary embolism.

(Dr. McCartney, closing.) There was no evidence of any seasonal distribution at all.

## PNEUMONIA IN NEWBORN INFANTS. Margaret Warwick, Buffalo, N. Y.

*Abstract.* In 420 consecutive autopsies on babies who were stillborn or died within the first 10 days of life, 32 were too macerated for detailed examination, but of the remainder, 77 or 18.7 per cent showed pneumonia. Of the 77, 27 or 48 per cent lived less than 24 hours, and 54 or 70 per cent lived less than 48 hours, showing that the condition was closely related to birth. All of the lungs were stained for bacteria which were present in 22 or 30 per cent. This number increased with age and, among those living less than 24 hours (37), only 3, or 8 per cent, showed bacteria, suggesting that they might be introduced after birth by artificial respiration or by aspiration. The possibility of bacteria having been introduced after premature rupture of the membranes was excluded by the fact that 49, or 63 per cent, had had the membranes ruptured less than 10 hours before birth, and 56, or 73 per cent, had ruptured less than 24 hours before.

The majority of these infants had had difficult births because only 17, or 22

per cent, had been born by a spontaneous delivery and of these, 5 had had unusually long labors. Only 11, or 14 per cent, were recorded as "good" at birth and 49, or 63 per cent, were dead or "poor" and the rest were "fair." Of the 77, only 16 died without other pathological lesions, such as malformations or birth injuries, sufficient to have caused death without the pneumonia. Therefore this pneumonia seems to be associated with the process of birth.

Infants frequently have premature respirations before or during birth due to asphyxia from some disturbance of the circulation of either mother or child, and this sucks amniotic fluid containing cornified epithelial cells and often bile pigments from meconium deep into the lungs. Of these 77 pneumonia cases 65, or 84 per cent, showed the cornified epithelial cells and bile salts to be present, while in the remaining 12, or 16 per cent, the pus cells were numerous enough to have obscured the evidence. So it seems that these irritating substances, which are foreign bodies, may set up an inflammatory process identical with a "chemical" pneumonia which would not have a toxic effect but would mechanically fill the alveoli and prevent proper aeration. An infant would probably have premature respirations during a long and difficult delivery and, if it were born in a poor condition, would lack the energy and vitality to expel the amniotic fluid so that much would remain to cause a pneumonia. A few may have the pneumonia already established at birth, if they have breathed long before.

Therefore it seems that the pneumonia which is found in about one-fifth of all infants dying during the first 10 days of life is the effect of a hazardous birth and not an infectious process and is more commonly found in weak infants, or those who have been maltreated during birth.

### *Discussion*

(Dr. Herbert S. Reichle, Cleveland.) Has Dr. Warwick any statistics as to whether there were any areas preferred by the process in those cases where the disease was not diffuse throughout the lungs?

(Dr. Max M. Strumia, Philadelphia.) I should like to ask what criterion was used to determine the presence of bacteria in the lungs? Did you use stained smears only?

(Dr. A. W. Wright, Albany.) I should like to ask a question very much like that of the previous speaker, namely, did Dr. Warwick carry out blood culture studies in the case of any of the infants, and if so, did such cultures show anything of significance? I also have a second question. If the pneumonic process was recognized at the time of gross examination of the lungs, were cultures from these organs taken at that time? Dr. Warwick's percentage of acute pneumonias in young infants is considerably higher than ours. We have on certain occasions found that the blood has contained pathogenic bacteria while there was no evidence at all of a pneumonic process. We have also seen, as Dr. Farber and his colleagues have, a considerable number of lungs that contained aspirated amniotic fluid and desquamated squamous epithelial cells without any evidence of a marked inflammatory reaction such as we saw in some of the photomicrographs shown by Dr. Warwick.

(Dr. Irving Graef, New York City.) I should like to ask if Dr. Warwick has taken into consideration the possibility that the polymorphonuclear leukocytes found in the lungs were also aspirated from the amniotic sac, reflecting inflammation of that structure. In the material at Bellevue Hospital from the obstetric and pediatric services, it is also common to find cases in which the

aspiration of amniotic fluid, indicated by the presence of epidermal cells and meconium, is not associated with polymorphonuclear leukocytes. Therefore I feel sure we are not justified in concluding that these normal elements of the amniotic fluid necessarily incite an inflammatory process.

(Dr. Warwick, closing.) In reply to Dr. Reichle, there seemed to be no preferred areas except around the bronchi, where we nearly always found the areas of pneumonia. We found many of them in the upper lobes and many in the lower, while a great many were diffuse throughout the lung.

Our only criterion for the presence of bacteria was staining, and we carried that through with controls. No blood cultures were taken, and no cultures were made of the lungs themselves. Unfortunately in our hospital it is difficult to get autopsies at once on these babies. We try to get permission for them, which is at first refused, and then all the way from 6, 8, 12 or even 24 hours later the families will consent to the autopsy, so we do not have proper culture material. But I have cultured several cases without finding anything.

These pneumonias were not recognized clinically in the majority of cases, because the pediatrician called them atelectasis. A few of them had X-ray pictures, and they noted what they called atelectasis, which gives the same X-ray picture as this pneumonia.

In regard to Dr. Graef's suggestion as to the possibility of the leukocytes coming from the amniotic fluid, we found a good many of these young infants in the series that showed aspirated amniotic fluid without showing pneumonia, that is, the criterion of the epithelial cells and the bile salts. These were very distinct; they were usually pretty large in size; they did not look like bacteria; they had very different shapes and a different color, and with the ordinary oil immersion lens it seems to me that the bile salts could not be mistaken for bacteria, or the other way around. It is certainly evident that we have large numbers of infants who aspirate amniotic fluid who do not show polymorphonuclears in the lungs, in the alveoli or bronchi, and it seems to me there is the possibility that had some of these infants had a little more strength to get rid of the amniotic fluid shown by the small areas of lung infected, and had they not had other hazards to their early life, they might very easily have not succumbed to this pneumonia. I do not think it is toxic; I do not say it is the cause of death, except from the mechanical point of view by filling up the lungs and preventing proper aeration. I will admit the bacteriological studies are not sufficient, but our stains for bacteria are satisfactory, and it seems very easy to recognize them, and as I said before, they were stained with controls from pneumonia in adults.

NEOPLASTIC CHANGES PRODUCED IN THE OVARY AND OTHER ORGANS OF MICE BY IRRADIATION OF THE ENTIRE BODY WITH X-RAYS. J. Furth and (by invitation) J. S. Butterworth, New York City.

*Abstract.* The incidence of most spontaneous neoplasms in mice is greatly increased by massive irradiation at the age of from 2 to 3 months; the incidence of breast cancer is decreased.

Lymphomatosis		Myelosis	Tumors				Negative
Mediastinal	All other types		Breast	Ovary	Lung	Miscellaneous	
Irradiated mice (374 ♀, 405 ♂):							
9.1 %	8.0 %	7.1 %	5.9 %*	17.4 %*	10.7 %	3.7 %	55.2 %
Control mice (708 ♀, 567 ♂):							
1.3 %	4.0 %	0.9 %	11.6 %*	1.1 %*	7.1 %	1.7 %	78.3 %

\* Of females: all other percentages are those of all mice.

The ovarian tumors are granulosa-celled, adenoma-like and lutein-celled growths. Some of them are associated with cystic hyperplasia of the endometrium (in 1 case, endometriosis), and hyperplasia of the anterior lobe cells of the pituitary (in 1 case adenoma-like).

### Discussion

(Dr. Kornel Terplan, Buffalo.) I should like to ask two questions about this very interesting demonstration: were there any hematological blood studies made on the mice previous to the appearance of the tumors? The reason I ask this question is because we may see in man following severe damage to the bone marrow a severe anemia, and almost complete disappearance of the granulated elements, which may later be followed by acute myelogenous or lymphatic leukemia. In one of these cases which I saw there was a combination of lymphatic leukemia with blastomatous proliferations of reticulum cells.

My second question is whether Dr. Furth or any other member of this Society ever happened to see radiation, especially roentgen-ray treatment of epithelial tumors, followed by the appearance of a sarcoma at or near the site of the apparently healed carcinoma. I remember a case with a primary sarcoma following many years of extensive X-ray treatment of a basal cell carcinoma of the face. This sarcoma was very close to a diffuse hyalinized scar which was the remainder of the X-ray-treated basal cell carcinoma.

(Dr. David P. Seecof, Montreal.) Are these animals which are irradiated and which develop tumors of the ovaries sterile, or do they ever have any litters? Is the X-ray dose sufficient to sterilize them for the remainder of their lives?

(Dr. Furth, closing.) In regard to Dr. Terplan's question, the dose used produces profound atrophy of the bone marrow with leukopenia. Leukemia and in general the tumors mentioned arise in organs that were severely injured by the X-rays. The appearance of neoplasms follows regenerative processes.

It is conceivable that radiation of a tumor produces another neoplasm, but one would expect the second neoplasm to appear after a very long incubation period. Long latency is characteristic of experimental cancer.

To answer Dr. Seecof's question, the ovary remains free from ova for the rest

of the animals' lives. This is significant in relation to the discussion whether there is postnatal ovum formation or not. We found excessive regeneration of all other elements of the ovary, but have never seen an ovum in irradiated ovaries.

THE CELL FACTORS INFLUENCING VIRUS-INDUCED PAPILLOMAS OF THE RABBIT.  
John G. Kidd (by invitation), New York City.

*Abstract.* The rabbit papillomas caused by the Shope virus are formed by the multiplication of the epidermal cells originally infected with the virus. These growths vary in their course, some enlarging progressively, while others retrogress. Both in their enlargement and retrogression the growths offer opportunities for the study of the part played by the cells in a prolonged virus-cell association that gives rise to growths of neoplastic character.

The activity of the virus material is of prime importance in determining the course of the growths, but host factors exercise an influence which is frequently decisive. Multiple papillomas produced with the virus in any one individual run the same general course when this is not complicated by the influence of intercurrent local factors, but from host to host the variation in size and fleshiness is considerable. The growths are generally larger and fleshier in rabbits whose skin proliferates actively in response to the injection of scharlach R, dibenzanthracene, tar extracts, and certain other stimuli. When virus suspensions from several different wild rabbits are tattooed into many spots on the skin of each of a group of comparable domestic rabbits, the growths that result from the different inoculums may differ considerably in time of incubation and rate of enlargement, yet those of a single host all tend to wax or wane together. In some animals they enlarge progressively, whereas in others they grow well for a time and then retrogress. Evidently some general influence of host derivation determines their fate. This influence is distinct, however, from that of the principle neutralizing the virus which appears in the blood of rabbits bearing the papillomas, for this principle does not influence the course of the growths significantly. Experiments have shown that the papillomas grow steadily in animals whose serums have marked capacity to neutralize the virus *in vitro*, and good evidence exists that the cells protect the virus stimulating them.

Many transplanted tumors call forth a resistance on the part of the host which is directed against the proliferating cells. Can it be supposed that a similar phenomenon is elicited by the papilloma during its growth, the resistance in this case being directed against the animal's own virus-infected cells? Certain facts speak in favor of this conception. In three groups of comparable domestic rabbits, 4, 24, and 64 discrete papillomas respectively were produced by tattooing virus into the skin of the sides. The incidence of retrogression was the same in all of the groups, but the larger the number of papillomas the sooner and more rapidly did it occur. Evidently the host's own proliferating, virus-infected cells, or some product of these, acted to elicit a host resistance, the effectiveness of which varied with the amount of papilloma tissue calling it forth. The resistance, however, was not always completely effective. In several exceptional instances small growths retrogressed completely, whereas larger ones of the same host, though dwindling when the others did, did not wholly disappear but ultimately enlarged again and later grew progressively. Whatever the nature of the defense mechanism responsible for retrogression, it is evidently subject to fluctuations in effectiveness.

In its histological features the retrogression of the papillomas resembled that of epidermal tumors generally, notably the tar papillomas — a fact which supports the view that it may be due to similar influences exerted upon the cells. The growth becomes more orderly, hair follicles and sebaceous glands reappear beneath it, its processes become narrower, and almost insensibly their place is taken by normal epidermis. Confluent papillomatous masses take much longer to retrogress completely than small discrete growths, the process going on principally at their periphery. Local stimulation of the papilloma cells by bacterial infection or scharlach R not only makes vigorous papillomas grow faster but may check and, for a time at least, prevent retrogression. The reactive inflammation present in the large masses acts to favor their progressive growth.

It seems likely that the host resistance which not infrequently overcomes rabbit papillomas is elicited by and directed against the animal's own proliferating virus-infected cells. The frequency of retrogression can be referred to the unfavorable character of many of the rabbits in which papillomas are experimentally induced.

**NESIDIOBLASTOMA, THE ISLET CELL TUMOR OF THE PANCREAS.** George F. Laidlaw and (by invitation) Virginia Kneeland Frantz, New York City.

*Abstract.* A study of nine adenomas of the islets of Langerhans removed surgically from six patients by Dr. Allen O. Whipple, at the Presbyterian Hospital, New York City, is presented. In each instance there followed prompt disappearance of the hypoglycemia with its collateral symptoms, for the relief of which the operations had been performed. The tumors were small, varying from 4 mm. to 2 cm. in diameter.

Microscopically the chief feature of most of the tumors is their exact duplication of the pattern of normal islets, consisting of longer or shorter ribbons of cells bordering on a rich plexus of capillaries. Cellular details are revealed best by fixing in Zenker's fluid, staining paraffin sections with acid fuchsin and differentiating in methyl green. In normal pancreas, so treated, the acinus cells are green with green nuclei, zymogen granules red, basal filaments and other mitochondria red. In contrast with the green acinus cells, the islet cells are packed with bright red granules. With a slight modification of the technique, the granules of the *A* cells hold the red; the granules of the *B* cells turn purple. The tumor cells react to this stain exactly like islet cells. In most of the tumor cells the granules take the purple color of *B* cells with here and there a red *A* cell. In the tumors, we have not found Bensley's granule-free *C* cells or the *D* cells which stain with aniline blue.

The tumors resemble islet hypertrophies in their tendency to exaggerate some feature of the normal islet pattern. For instance, Tumor 2 repeats over and over and exaggerates the rosette formation around the blood channels which is an occasional figure in normal islets. Tumors 5 and 6 consist almost entirely of long ribbons of large columnar cells with centrally placed nuclei, exaggerating and repeating a feature sometimes seen in normal islets, but more frequently in their hypertrophies. Tumor 1 copies the usual compact endocrine pattern so exactly that even with the highest magnifications one cannot distinguish it from a normal islet except at the margin where it compresses the surrounding pancreas, and from its size. It is really one gigantic islet 1 cm. across.

Just as the tumors duplicate the structure of normal and hypertrophied islets, so are they subject to the same pathological vicissitudes, especially fibrosis,

hyaline degeneration and calcification. We agree with other observers that the fibrosis begins along the capillaries as a fibrous thickening of the capillary wall with projection of small blocks of collagen encroaching on the tumor cells. Six of our tumors show extensive fibrosis. Much of the newly formed fibrous connective tissue has been converted into a clear glassy substance which, in the negative outcome of amyloid and mucin reactions, we must be content to call hyalin.

With Mallory's aniline blue connective tissue stain or with its variants, Masson's trichrome and Heidenhain's azocarmine, the hyaline substance stains pale blue, much paler than the fibrous connective tissue. Many tumor cells show similar pale blue patches in their cytoplasm. Studying these patches, we are convinced that the tumor cells themselves undergo the hyaline change as well as the fibrous connective tissue, settling in our own minds at least the long-standing controversy as to whether the hyaline metamorphosis is restricted to the collagen or to the cytoplasm. It affects both.

In some of our tumors the origin of the tumor cells is indicated by figures where the epithelial lining of a duct is continuous with a group of tumor cells. Here again we observe an exaggeration of a normal procedure. In the normal pancreas, continuity of duct cells and islet cells is a common observation. It is admitted by all that the duct cells are totipotent, producing acinus cells and islet cells during embryonic life and probably throughout adult life as well. If the cells of the islet tumors differentiate out of duct epithelium, they are merely exaggerating the normal procedure of islet building, probably with coincident formation of many new ducts.

The name *nesidioblastoma*. There is need for a short and accurate name for these tumors. Adenoma of the islets of Langerhans is long and cumbersome. Adenoma itself is vague, for we have already two kinds of adenoma, the benign epithelial tumor and lymphadenoma, quite different from each other. Another adenoma, an endocrine kind, merely adds to the confusion. We have followed current custom of suffixing "oma" to the Greek name of the cells of origin of the tumor. Selecting *νησίδιον* as the Greek word for islet, the cells that differentiate out of the duct epithelium to build islets may be called nesidioblasts, or islet builders. When these islet builders or nesidioblasts form tumors, the tumor is a nesidioblastoma. The name has another application. In contrast with the concentration of excess islet tissue in a tumor, evidence is accumulating of a diffuse or disseminated proliferation of islet cells as a possible cause of hypoglycemia. Such a proliferation of nesidioblasts would be a nesidioblastosis.

**LIPOSARCOMA OF BONE: A REPORT OF TWO CASES.** Donald J. Rehbock and Harry Hauser (by invitation), Cleveland, Ohio.

*Abstract.* The 1st case presented is that of a woman 56 years of age who had a pathological fracture of the right femur, followed in 7 months by a pathological fracture of the left femur. The patient died 15 months after the onset, and was found at autopsy to have generalized tumor metastases, the primary site being in the right femur. The histological diagnosis was liposarcoma.

The 2nd case was that of a 60 year old white man who was seen only shortly before death, and who had had symptoms for 1 year. At autopsy there was found a large tumor involving the right ilium and adjacent sacrum. There were no tumor metastases. The histological diagnosis was liposarcoma.

These cases are No. 1904 and No. 1224, respectively, of the American College



of Surgeons Bone Sarcoma Registry. A review of the literature reveals only 5 other recorded cases of primary liposarcoma of bone.

### *Discussion*

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Rehbock how he distinguishes this neoplasm from the Ewing tumor of bone. It has the same general features as a Ewing tumor. The fat in the cells might be merely a degenerative process which we often see in malignant tumors. There is no direct evidence of a differentiation into adipose tissue cells.

(Dr. S. Burt Wolbach, Boston.) I was about to suggest that tumors be stained with fat stains more frequently, because I think we would find fat much more often than expected. That has been our experience with some tumors that obviously could not be regarded as lipomas — including epithelial tumors.

(Dr. Kornel Terplan, Buffalo.) I should like to ask whether one of the tumors shown could not be called a reticulum cell sarcoma. This type is not uncommon as a primary blastoma of the bone marrow. The small fat droplets which were seen in giant cells may have been phagocytosed from the fatty marrow in the bone.

(Dr. Rehbock, closing.) The diagnosis of liposarcoma here does not rest entirely on the presence of fat within the cytoplasm of the cells. The cells must show no evidence of nuclear or cytoplasmic degeneration. It is true we did find fat in areas which obviously show degeneration, but we did find fat in the undegenerated cells. The cellular morphology is much different from that of the Ewing tumor and reticulum cell sarcoma. The morphology of the cells, I believe, is a more important clue to the diagnosis than the presence of fat, — large cells with abundant cytoplasm, resembling the embryonic type of fat cell.

### BONE MARROW PICTURES IN THE ANEMIAS AS STUDIED BY STERNAL BIOPSY. R. P. Custer, Philadelphia, Pa.

*Abstract.* Biopsy of the sternal bone marrow has been practiced with increasing frequency at the Philadelphia General Hospital during the past 4 years and has served (1) to give the patient the benefit of more accurate diagnosis and prognosis; (2) to afford the attending clinician firmer ground on which to base his therapeutic measures; and (3) to catalogue more adequately the various disorders of the blood-forming organs and accumulate further data for the study of these disturbances.

A 1 cm. diameter button of bone is removed from the ventral table of the sternum by trephine under local anesthesia; imprints and streaks are stained by Pappenheim's May-Grünwald-Giemsa method. The button is fixed in Helly's fluid, decalcified in formic acid-sodium citrate solution, sectioned in paraffin at 4 microns and stained by a modification of Maximow's azure II eosin method. Differential counts of 500 cells are made by the method previously described (Krumbhaar and Custer).

A small chapter from the accumulated data is presented, depicting differences and similarities of the bone marrow picture in a few selected types of anemia as shown by matched photomicrographs and differential counts. Abbreviated counts from the group follow:

	Hemorrhagic anemia	Pernicious anemia			Normal	Idiopathic aplastic anemia	Idiopathic hypochromic anemia	Parasitic anemia (uncinariasis)	Sickle cell anemia
		Relapse	Early remission	Late remission					
UNDIFFERENTIATED CELLS	1.8	2.4	0.6	0.4	0.0	0.8	2.6	1.2	2.4
MYELOBLASTS	0.2	0.8	1.0	1.4	0.6	0.0	0.8	0.4	0.8
PROMYELOCYTES	2.4	0.8	1.6	6.6	9.0	0.4	1.8	1.8	1.8
MYELOCYTES	0.0	0.0	0.4	0.6	0.0	0.0	0.0	1.2	0.0
	5.4	3.4	4.0	15.4	34.6	0.8	7.4	6.0	8.2
	1.6	2.8	3.0	2.0	2.0	0.2	0.8	5.2	0.8
METAMYELOCYTES	8.4	4.6	5.8	9.2	14.6	1.1	9.8	9.0	10.8
	0.8	1.2	2.6	1.0	0.0	0.2	1.2	2.6	0.4
SEGMENTERS	3.4	10.2	8.4	6.8	2.9	0.0	5.4	5.4	4.8
	0.2	3.2	1.8	0.8	0.1	0.0	0.0	1.6	0.2
TOTAL	22.4	27.0	28.6	43.8	63.8	2.7	27.2	33.2	27.8
PROMEGALOBLASTS	1.8	2.8	1.0	0.8	0.0	0.2	4.4	4.6	1.2
MEGALOBLASTS	0.6	38.6	8.2	3.6	0.0	0.4	3.8	3.2	2.4
ERYTHROBLASTS	33.6	13.8	47.2	22.2	14.8	45.2	34.4	31.8	33.4
NORMOBLASTS	32.0	5.4	10.1	27.2	18.2	30.4	20.2	23.2	24.0
TOTAL	68.0	60.6	66.5	53.8	33.0	76.2	62.8	62.8	61.0
MEGAKARYOCYTES	1.2	0.8	0.8	1.0	1.0	0.2	0.4	0.4	0.8
RETICULAR CELLS	3.2	5.2	1.6	0.8	1.2	11.0	5.0	1.4	4.0
ENDOTHELIAL CELLS	3.4	4.0	1.6	0.2	1.0	9.2	2.0	1.0	4.0

Biopsy should be performed whenever possible before treatment of any kind has been instituted, especially liver administration. This is well shown by the example of early remission in pernicious anemia in which the patient was given a single dose of liver extract parenterally 2 days before biopsy; striking changes in the marrow obviously precede changes in the peripheral blood.

While a scattering of promegaloblasts and megaloblasts appears in the marrow in practically any type of severe anemia, erythropoiesis is essentially of the so-called normoblastic type in all varieties shown here except pernicious anemia in relapse; in aplastic anemia a great gap lies between stem cells and late nucleated red cells, the few megaloblasts being confined to tiny foci of attempted regeneration. In sickle cell anemia erythropoiesis is normal save for a very occasional sickled normoblast.

While the bone marrow picture may occasionally be inconclusive, it is much less often so than studies on the peripheral blood alone, and the practice of sternal biopsy deserves wider use than it is now accorded. Emphasis is again laid on the value of carefully prepared sections in conjunction with smears, as opposed to study of smears obtained by simple sternal puncture.

### Discussion

(Dr. S. Burt Wolbach, Boston.) I must say I congratulate Dr. Custer on the size of the trephine that is used in Philadelphia. I wish we could persuade them to use similar trephines in Boston hospitals.

### THE PRODUCTION OF PERSISTENT HYPERTENSION IN MONKEYS. Harry Goldblatt, Cleveland, Ohio.

*Abstract.* By the constriction of the renal arteries by means of a special silver clamp, smaller than the type which was originally devised for dogs, a method already reported, experimental hypertension has been produced in monkeys (*Macacus rhesus*). For months before and after the production of bilateral renal ischemia, systolic and diastolic pressures were determined by the Riva Rocci method (the cuff being applied around the lower part of the abdomen) with auscultation over the femoral artery. Systolic and diastolic pressures became greatly elevated following constriction of the renal arteries and a corresponding elevation of "mean blood pressure" was also found by direct puncture of the femoral artery and connection with a mercury manometer. In one monkey the hypertension has now persisted for 13 months. During this entire period the average systolic pressure has been about 150 mm. Hg. higher and the average diastolic pressure about 100 mm. Hg. higher than during the control period. The "mean blood pressure," as determined by puncture of the femoral artery and direct connection with a mercury manometer, also showed an increase of 150 mm. Hg. over the control period.

### SPLANCHNIC SECTION IN EXPERIMENTAL HYPERTENSION. Harry Goldblatt and (by invitation) Jerome Gross and Ramon F. Hanzal, Cleveland, Ohio.

*Abstract.* The purpose of this investigation was to determine whether, in dogs, splanchnic section would prevent the elevation of blood pressure produced by renal ischemia or lower the pressure in dogs with experimental renal hypertension. The lower portion of the dorsal sympathetic chain, including about four

ganglia, and the thoracic portion of the splanchnic nerves were excised by the intrathoracic route. In four normal dogs, after a variable period following this operation, the renal arteries were constricted moderately by the application of an adjustable silver clamp, a method previously described. The systolic pressure rose, as usual, in all four animals and renal function tests showed little or no impairment. In two other dogs, in which the constriction of the arteries was purposely made severe, the blood pressure rose but there was severe impairment of renal function, clinical uremia developed and the animals died in a few days. Excision of the lower dorsal sympathetic ganglia and the thoracic portion of the splanchnics was also performed in four dogs with experimental renal hypertension that had existed for from a few weeks to 4 years. In none of these animals was there any significant change in the blood pressure following the splanchnic section. After excision of the nerves the blood pressure fell temporarily in some of the animals but it soon returned to the original level. It appears, therefore, that in the pathogenesis of experimental renal hypertension due to renal ischemia the vasomotor mechanism of the splanchnic region plays little if any part. These results fail to give experimental support for the operation of splanchnic section that is now being practiced on human beings with hypertension.

#### *Discussion*

(Dr. E. T. Bell, Minneapolis.) We are very much indebted to Dr. Goldblatt for this splendid way of producing persistent hypertension in animals. It gives us an opportunity to do a lot of experiments which may help us to understand the fundamental problems of hypertension. It appears from his work that hypertension due to disease of the kidneys is not a reflex phenomenon. A great many investigators have held that obstruction in the renal circulation gives rise to a reflex through the sympathetic which brings about the rise in blood pressure. It is quite clear that this is not the true explanation, and we have to proceed now to find out by what mechanism the blood pressure is increased. I want to ask Dr. Goldblatt if he thinks this obstruction of the arteries might not be analogous to congenital stenosis of the aorta which we see so frequently. A person may live to adult life with a stenosis of the distal part of the arch and show hypertension in the upper part of the body and hypotension in the lower extremities. That goes on to a marked hypertrophy of the heart and death from heart failure or rupture of the aorta in many instances. Is it possible that this obstruction of the renal arteries is the same kind of a process as congenital aortic stenosis? There is another line of study which may be used here: the effect of prolonged hypertension on the smaller arteries throughout the body. I think Dr. Goldblatt should carry on this work and see if he can find arteriosclerosis in the various organs. One of the young men at Minnesota has used his method, and I have studied the kidneys of animals which have had this type of hypertension for 3 to 4 months, and the arterioles showed no changes in these animals. Possibly the time elapsed is not long enough, or it may possibly be because the pressure is not so elevated in the renal vessels.

(Dr. Goldblatt, closing.) I do not believe that the hypertension of congenital aortic stenosis is directly comparable to the experimental renal type of hypertension, for at least two reasons. (1) In the case of aortic stenosis, the mechanical factor *per se*, by interfering with the onflow of blood, plays a primary part in the pathogenesis of the hypertension; whereas, in renal hypertension, it is the *renal ischemia* produced by the mechanical factor which is of primary importance.

(2) It has been shown by Prinzmetal and Wilson that the vasomotor apparatus plays an important part in the continuance of the state of hypertension associated with aortic stenosis, whereas the experiments reported here indicate that the vasomotor mechanism is of relatively little, if any, importance in experimental renal hypertension.

The possible effect of long continued elevation of blood pressure on a vascular system that was normal prior to the production of the hypertension is, of course, of great interest to us. Some of our animals have had hypertension for about 5 years. We hope as a result of study of such animals to be able to answer Dr. Bell's question about the effect of the hypertension on the vascular system subjected to this elevated pressure. The changes, if any, in the vessels within the kidneys would obviously be due to *hypotension* and ischemia rather than hypertension and would not be comparable to any changes found in the rest of the vascular apparatus. It is difficult to decide when to terminate the experiments. A negative result, that is, no pathological change in the blood vessels, would be of significance only if the hypertension had existed for a long time. On the contrary, pathological changes found in old animals with experimental hypertension might be attributable to the age factor alone. We shall be obliged to study the possible effect of various periods of hypertension in different animals. Changes due to the hypertension ought to progress with the length of time during which the vessels are subjected to the elevated pressure. We are not yet in a position to give an answer to this question. I am very pleased that Dr. Bell is using our method for the production of hypertension.

THE EARLY STAGES OF GLOMERULONEPHRITIS. E. T. Bell, Minneapolis, Minn.

*Abstract.* A microscopic study of the kidneys was made in 107 cases of death from accidental causes, in 194 cases with death from non-infectious diseases, and in 564 cases of death from various infectious processes.

In the 107 normal kidneys the glomerular epithelial cells definitely outnumbered the endothelial in 84.1 per cent, the endothelial outnumbered the epithelial cells in only 1 instance (0.9 per cent), and the two types of cells were approximately equal in number in 15 per cent. It was concluded that a definite preponderance of endothelial over epithelial cells represents a glomerulitis.

A Grade 1 glomerulitis was found in 14 per cent of non-infectious processes.

In lobar pneumonia glomerulitis was found in only 18.8 per cent, but in the other infectious groups it varied from 37.5 to 78.9 per cent.

The highest incidence of glomerulitis was found in puerperal sepsis (52.4 per cent) and subacute bacterial endocarditis (78.9 per cent).

It is evident that a variety of toxic substances, especially those derived from streptococci, may irritate the glomerular capillaries and produce an increase of endothelial cells.

In a Grade 2 glomerulitis the glomerular capillaries are filled with cells and occasionally a few intracapillary fibers are present. The distinction from clinical glomerulonephritis is somewhat arbitrary.

The glomerulitis is probably due chiefly to endothelial proliferation, but the lodgment of mononuclear leukocytes in the capillaries seems to play a rôle of some importance.

There is no relation between the presence or the amount of albumin in the urine and the degree of endothelial proliferation.

There is no anatomical basis for a diagnosis of focal glomerulonephritis except

in instances of transitory glomerular bleeding, not associated with symptoms of nephritis, and in cases of bacterial endocarditis.

### *Discussion*

(Dr. Paul Klemperer, New York City.) I wonder, Dr. Bell, what you think of the possibilities of the intercapillary glomerulitis — this may be digressing a little bit from your topic — but I wonder how you feel about the intercapillary glomerulitis which was stressed recently by MacCallum and Kimmelstiel. Might not some of the cells which you could not place be fibroblasts?

(Dr. Bell, closing.) I have seen no evidence of intercapillary glomerulitis in this group. I have, however, seen intercapillary changes in some hypertensive forms of kidney disease.

RENAL LESIONS IN EXPERIMENTAL BENCE-JONES PROTEINURIA. H. Edward MacMahon and (by invitation) A. Magnus-Levy, Boston, Mass.

*Abstract.* There are several interesting questions dealing with the relation between the elimination of Bence-Jones protein and renal injury that are still unsettled. Can Bence-Jones protein pass through a healthy kidney? Where in the kidney is Bence-Jones protein eliminated? Is Bence-Jones protein injurious to the kidney, and if it is, what is the nature of this injury?

Preliminary to our attempt to answer these questions we sought a suitable stain that would enable us to recognize Bence-Jones protein within the tissue. We found that smears made from concentrated solutions of Bence-Jones protein, as well as the pure crystals of Bence-Jones protein isolated from the urine, stained a brilliant red with acid fuchsin and a brilliant yellow with orange G. By combining these stains, as is done in the Mallory aniline blue connective tissue stain, the crystals stain a brilliant orange gold regardless of whether the fixative used was Zenker or formalin solution. It was also observed that the large crystals which are sometimes found lying freely within the substance of myeloma tumors — and regarded as Bence-Jones protein — stained in an identical manner. The abundant precipitate which is so often found within the renal tubules, and the crystals that are occasionally found in the same tubules in cases of Bence-Jones proteinuria, behave with this stain in a similar way. Although this stain, in a chemical sense, is not absolutely specific for Bence-Jones protein, it offers a very good indicator when used with other stains for the presence of this material.

In 1931 we began this study of the renal changes following subcutaneous and intraperitoneal injections of varying quantities of Bence-Jones protein.

Sixty white mice in all were used in this experimental study. The animals were divided into two groups. The animals of Group 1 received a single injection (0.1 gm.) of a 5 per cent solution of Bence-Jones protein. These mice were autopsied at 2, 4, 6, 8, 16 and 24 hour intervals and at intervals of 2, 4, 6 and 8 days. The animals of Group 2 received daily injections during a period varying from 1 to 6 weeks.

*Group 1:* (Mice of this group had received a single injection.) After 2 and 4 hours no changes are recognizable in the kidney. As early as 6 hours the cells of the distal convoluted tubules show at their bases a brilliant orange granulation of their cytoplasm, leaving the cytoplasm adjacent to the lumen unchanged. At this time there is no recognizable change in the glomerulus or

in the vessels, and the tubules are empty. At the end of 8 hours these same cells of the distal convoluted tubules are shrunken and diffusely and brilliantly stained, making a sharp contrast to all of the cells of the remaining tubules. In the hematoxylin and eosin stained preparations these changes are scarcely recognizable. The glomerulus and vessels are unchanged, and the capsular space of the glomerulus and the lumens of the tubules are empty. After 16 hours these same cells, singly and in chains, are desquamated. They stain intensely a brilliant orange gold with the Mallory connective tissue stain, and lie freely within the tubule. The lumens of the tubules and the glomerular spaces contain a hyaline substance staining as Bence-Jones protein. At the end of 24 hours desquamated epithelial cells are still recognizable, and tubules here and there contain a small amount of material resembling Bence-Jones protein. The most striking change at this time is the early sign of regeneration, for mitoses in the epithelial cells are numerous, and the denuded areas within the tubules are being relined. At the end of 1 week the kidney shows no pathological change and Bence-Jones protein is no longer to be found.

In brief, it does appear from these experiments that Bence-Jones protein may pass through a healthy kidney and that for the most part it is eliminated through the glomerular tuft. Furthermore, it is seen to injure the epithelial cells in a distinct segment of the tubule, leading to death of these cells and ultimate desquamation. The injury is simply one of degeneration, which is quickly followed by a process of regeneration without vascular changes of inflammation. It would seem from the very early appearance of the Bence-Jones protein within the tubular epithelium — before it is found within the glomerular capsule or in the lumen of the tubule — that this protein may be eliminated by the tubular epithelium as well as by the glomerular tuft. Bence-Jones protein may fill the lumens of the tubules without producing any apparent injurious effect on the majority of cells lining the tubules, leading one to believe that as long as it lies outside the cell it is comparatively innocuous, whereas as soon as it is taken up by the cell it leads to regressive changes which may result in necrosis. The lesion that is produced in the kidney following a single subcutaneous injection of this protein is completely reparable.

*Group 2:* (Mice of this group had received successive daily injections.) At the end of 48 hours the entire tubular structure of the kidney with the exception of the collecting tubules shows a severe degenerative change. The cells of the proximal convoluted tubules immediately associated with the glomerulus are large, swollen and vacuolated and the cytoplasm is scattered, rather clumped, and granular. These cells do not contain Bence-Jones protein. The next group of cells of the proximal convoluted tubule, as one passes down the nephron, are even more swollen and, in the hematoxylin and eosin stained preparation, show the most extreme form of hyaline droplet degeneration. The cell boundaries are indistinct and the inner cell boundary lining the lumen is incomplete and broken. The cells in places are so swollen and the cell membranes so indistinct that here and there it is impossible to distinguish the lumen or to make out the histological structure of the tubule. For the most part these droplets are largest toward the lumen, whereas the cytoplasm at the base of the cells is dust-like and granular. At times it is impossible to say whether the droplets are within the cytoplasm of the cell or within the lumen of the tubule because of the interruption of the lining cell membrane. In preparations stained with the aniline blue connective tissue stain these droplets stain as Bence-Jones protein. The lumens of the tubules and

the capsular spaces contain a similar material. The glomerular tuft, the blood vessels and the basement membrane of the tubules are unchanged.

After 2 weeks the histological picture has changed. Droplet degeneration is still present in many of the proximal and distal convoluted tubules. Bence-Jones protein is present within the collecting tubules, and the droplets in the tubules stain, as before, as Bence-Jones protein. The most interesting change at this period is the presence of many tubules lined with young regenerated cells showing here and there mitoses. The cells lining these tubules are flat, rather small and appear undifferentiated. Although Bence-Jones protein is present in the lumens bordered by these cells, their cytoplasm is unchanged. At this period there is very slight and somewhat focal contraction of the cortex. The glomeruli and the blood vessels are still unchanged.

At the end of 4 weeks and at the end of 6 weeks changes are first observed in the kidneys grossly. The surface shows a very fine granulation. Microscopically the reconstruction of the cortex is quite obvious and the sclerosing process is more marked. Some of the tubules are dilated, others are collapsed and bordered by an increase in connective tissue. Mitotic figures in the epithelial cells lining the tubules are occasionally seen. Casts of Bence-Jones protein are found within the tubules. The collecting tubules remain unchanged and there is no dilatation of the tubules proximal to the casts within the lumens. Here and there a glomerulus in one of the areas of scarring is small, rather collapsed and free of blood while the capsule and pericapsular tissue are thickened. The blood vessels throughout the kidney show no changes.

In summing up our observations based on this second group of experiments in which Bence-Jones protein was repeatedly injected, it does appear that the kidney suffers a permanent injury, most marked in the tubules with less extensive injury to the glomeruli. The damage is one of degeneration of the epithelial cells and, as in the first group of experiments, the outstanding features of an inflammatory reaction are absent. Degeneration and regeneration with a reconstruction of the kidney take place simultaneously.

*Summary:* Bence-Jones protein may pass through a healthy mouse kidney. For the most part it is eliminated from the body by way of the glomerular tuft, though there is reason to believe that the tubule may take part in this elimination as well. Bence-Jones protein, when taken into the cytoplasm of the cell in sufficiently large quantities, exerts an injurious effect leading to regressive changes and ultimate necrosis. The change within the kidney affects primarily the tubules and to a lesser degree the glomeruli. The change is essentially one of degeneration. Following a single injection the lesions within the kidney are completely reparable, whereas in the course of successive injections the injury becomes permanent, resulting in sclerosis with contraction. The lesion may be regarded as a form of *nephrosis* and in the late stages where contraction has begun it may be considered a nephrotic form of renal sclerosis.

Realizing that other factors may play an important rôle in the histological changes in the kidney in human cases of chronic Bence-Jones proteinuria, this does not militate against the experimental facts that Bence-Jones protein alone may produce serious renal injury.

#### *Discussion*

(Dr. Wiley Davis Forbus, Durham, N. C.) We too have been interested in the excretion of Bence-Jones protein by the human and the animal kidney, and have



reported experiments which are quite similar to those Dr. MacMahon described. We used three types of animals — dogs, rabbits and white mice. In none of the animals were we able to see that the excretion of relatively large quantities of protein over a considerable period of time had any effect whatever on the kidney epithelium. We were unable to produce in any of these three types of animals a lesion which we thought at all comparable to that which is commonly found in the human kidney when relatively large quantities of the protein are excreted. We too were very much interested in the possibilities of identifying Bence-Jones protein by means of characteristic and specific staining reactions, but we never succeeded in convincing ourselves that this could be done by means of the numerous stains which we used. We followed our animals for a considerable period of time. We also controlled the lesions in the kidneys by preliminary removal of kidney tissue. We did not, as Dr. MacMahon did, examine the kidneys of our animals shortly after the administration of protein, but our animals were excreting Bence-Jones protein when they were sacrificed. We have no information as to what might happen within a short time, let us say a matter of a few hours after the substance is given. It seems rather strange that injuries which are so pronounced as those described by Dr. MacMahon did not leave some permanent effect in the kidney in our animals, assuming that the protein had produced such injury immediately following the administration.

(Dr. J. Furth, New York City.) I wonder if Bence-Jones protein is antigenic and, if so, whether it would not be expected that in a heterologous species it would provoke a reaction that would not occur in a homologous species.

(Dr. MacMahon, closing.) I wish to thank Dr. Forbus for discussing this paper in such detail. I am very familiar with his paper which appeared only a few months ago. I wonder if by chance the apparently contradictory results of his experiments could be explained in part either by a difference in quantity of the material injected or by a variation in the concentration of the material injected. In our experiments minute quantities failed to produce recognizable changes, and equally important is the fact that when concentrated emulsions of Bence-Jones protein were injected subcutaneously it remained localized in the tissues for some time. The protein set up an inflammatory reaction about the deposit, but produced no significant renal changes. In contrast with this finding, we repeatedly observed that when a moderate amount of this protein was injected in dilute solution subcutaneously it appeared very soon in the urine and produced striking regressive changes in the tubular epithelium and less marked changes in the glomeruli.

In regard to the specificity of the stains, I do not believe that from a chemical standpoint the Mallory connective tissue stain is specific for Bence-Jones protein. Serum globulin and fibrinogen, for example, stain in a somewhat similar way, but it does appear that if used with other stains the Mallory connective tissue stain offers the best method of demonstrating this protein within the tissues.

This protein is antigenic. In our control experiments other human serum proteins were used without producing similar reactions.

**AMYLOIDOSIS WITH UNUSUAL DISTRIBUTION AND BENCE-JONES PROTEIN.** H. E. Robertson and (by invitation) L. A. Brunsting, Rochester, Minn.

*Abstract.* In the case of a man 44 years of age, who was otherwise in good health, muscular pains and weakness, particularly in the shoulder girdle, and yellowish nodules on the skin, especially of the eyelids and face, developed over a period of

2 years. The nodules were deeply colored by Congo red. The patient's urine contained Bence-Jones protein. No traces of myeloma could be found. At autopsy, in addition to the corium, amyloid was found in the tongue, walls of the esophagus, stomach, small intestine, colon and bladder. Mere traces were present in the kidneys, liver and spleen. A softened marrow in a vertebra was the only sign of a lesion to be found in the bones: this marrow contained a large increase in plasma cells, suggesting a cryptic origin for the proteinuria.

### *Discussion*

(Dr. Shields Warren, Boston.) I should like to ask Dr. Robertson whether the presenting symptoms in this case were the cutaneous nodules, or the involvement of the tongue. In 2 somewhat similar cases we have had, not so carefully studied unfortunately, we made the diagnosis in the 2nd case from the involvement of the tongue. The patients in both instances complained that their tongues were too large for their mouths, they had difficulty in swallowing, and their tongues had the characteristic rubbery feeling of the amyloid spleen or liver. I wonder whether in view of the amount of amyloidosis in the tongue in Dr. Robertson's case the same finding occurred.

(Dr. H. E. MacMahon, Boston.) One of the most striking features of amyloidosis associated with multiple myeloma is the localization in which the amyloid is deposited. In contrast with the usual forms of amyloidosis associated with chronic suppuration in which the amyloid is confined primarily to the liver, kidney, spleen, adrenal and intestine, one finds it deposited in large masses in the bone marrow, in muscles, in cartilage, in connective tissue and even in fat tissue in cases of multiple myeloma associated with Bence-Jones proteinuria. The distribution of the amyloid in the case reported by Dr. Robertson follows more closely to the rule than to the exception for this group of amyloid diseases. In our experimental work in which Bence-Jones protein was injected over a long period of time in animals, we had hoped to find some trace of amyloid in the tissues. In no case, however, could we demonstrate experimentally the conversion of Bence-Jones protein into amyloid material. It is of interest that both Bence-Jones protein and amyloid are stainable with Congo red and if this were the only stain used for the demonstration of amyloid the results could be open to criticism, as this dye stains other forms of hyaline and colloid material as well.

(Dr. Robertson, closing.) The symptoms were not referable to the tongue in this case. The man complained of pain, particularly along the shoulder and hip girdles, and he also exhibited weakness which almost reached a myasthenia gravis type and probably resulted from an infiltration of the muscular bundles.

**"FIBRINOID" LESIONS IN ATHEROSCLEROSIS AND SYPHILITIC AORTITIS AND THEIR RELATION TO THROMBUS FORMATION IN THE AORTA AND CORONARY ARTERIES.** Eugene Clark (by invitation), Irving Graef, and (by invitation) Herbert Chasis, New York City.

*Abstract.* The term "fibrinoid" has been employed by many in designation of a substance encountered in the walls of affected arteries, which in its tinctorial behavior resembles fibrin. Mallory has described the occurrence of fibrin-like material in the intimal plaques of atherosclerosis and syphilitic aortitis, and has expressed the belief that this substance represents organizing fibrin. Recently, however, a different interpretation of the nature and source of this material has appeared. Yager believes that the fibrin staining material in the intimal aortic

plaques of atherosclerosis and syphilitic aortitis represents degenerated or necrotic fibrous tissue, and Leary has expressed a similar opinion of the fibrin staining material in the fibrous regions of the intimal plaques in coronary thrombosis. The subject has gained new and wider importance by their assertion that thrombosis may be brought about in such vessels when fibrinoid necrosis extends to the surface of the plaques.

This report is based on the study of autopsy material from the Bellevue Hospital. It comprises an examination of the plaques of 40 atherosclerotic and 38 syphilitic aortas in which thrombosis was not grossly visible. Proceeding from these observations we have considered the lesion in the intimal plaques of 9 parietal aortic thrombi and of 11 instances of coronary thrombosis, the latter studied by serial sections. In those instances where material resembling fibrin appeared in the intimal plaque, successive sections were stained by hematoxylin and eosin, Weigert's elastic tissue stain, Van Gieson's, Mallory's phosphotungstic acid hematoxylin, Gram-Weigert, the silver impregnation method of Foot and Foot, and the benzidine stain for hemoglobin. Though the tinctorial reaction of the material to gentian violet was not constant, the fibrinous material of the thrombi exhibited a similar inconstant behavior. With the other methods employed no essential difference in staining reaction could be discerned between the material within the plaque and the fibrinous component of thrombi.

In 17 of the 38 specimens of syphilitic aortitis, fibrin staining material, generally of homogeneous character, was found in the fibrous regions of the intimal plaques. In the majority of instances the fibrin staining material appeared as horizontal or oblique bands on the surface and within the superficial fibrous regions of the plaques. Young fibroblasts and argyrophilic fibrils were frequently observed in intimate relation to the fibrin staining masses; regressive or inflammatory changes were absent. In the fibrous regions of the intimal plaques of atherosclerotic aortas similar lesions were found in 15 of the 40 specimens studied. They were found in fibrous plaques, in the fibrous covering of non-ulcerated atheromas, and in the fibrous regions of ulcerated plaques. In parietal aortic thrombi, fresh thrombi were frequently deposited on a layer of dense homogeneous fibrin staining material, which was continuous with oblique and horizontal bands of similar tinctorial properties in the superficial fibrous regions of the underlying plaque. Similar findings were encountered in thrombosed coronary arteries, though more commonly in such vessels the fibrin staining material in the plaque was confined within lipoid zones.

The gradual transition from masses which are clearly surface deposits to those covered by endothelium and collagenous tissue with young fibroblasts yields strong support for the belief, first expressed by Mallory, that these fibrin staining bands in fibrous plaques represent repeated surface deposits that have undergone partial organization. In other instances, of less common occurrence, the findings suggest that a break in the collagenous covering of an atheroma has permitted penetration and dissection of the plaque by blood elements.

If by thrombosis we mean the deposition of blood elements on the vessel wall, then these fibrin staining bands in most instances represent the remnants of organizing parietal thrombi, which have become covered by connective tissue. On such laminated, condensed, and partially organized surface deposits a fresh thrombus of formed elements and orthodox configuration frequently supervenes.

In the coronary arteries, in some instances, thrombosis represents a slowly progressive process in which the repeated parietal deposition of blood elements

is followed by partial organization which may lead to an increase in the size of the plaque and a progressive stenosis of the vessel. Such organizing parietal thrombi may form the base on which a fresh occluding thrombus is deposited. In other instances coronary thrombosis occurs suddenly, an occluding thrombus forming on an ulcerated atheromatous plaque into which penetration of blood elements has occurred.

Finally, we could find no evidence to support the view that the deposits of fibrin staining material in the intimal plaques of atherosclerosis or syphilitic aortitis represent altered collagenous fibers. The tinctorial properties of this so-called fibrinoid material are attributable to its fibrinous component, though other blood elements which have lost their recognizable character may possibly share in its composition.

### *Discussion*

(Dr. Ralph D. Lillie, Washington, D. C.) May I ask whether in the course of these studies the relation of these fibrinous masses to the internal elastic membrane satisfactorily stained was demonstrated. I would feel more convinced if that technical procedure had been used, although the presentation sounds very convincing in any case.

(Dr. Clark, closing.) In answer to Dr. Lillie's question, successive sections were stained by the methods reported, which included the Weigert elastic tissue stain combined with the Van Gieson. The elastica was clearly outlined in these sections and separated the thick intimal plaque from the media. These fibrinous masses were always found in the superficial regions of the plaque, and they appeared lemon yellow with this stain.

ARTERIOSCLEROSIS: A COMPARISON OF THE PATHOLOGY AND CONSEQUENCES IN THE NEGRO AND WHITE RACES. G. H. Hansmann, Milwaukee, Wis., and (by invitation) J. R. Schenken, New Orleans, La.

*Abstract.* A review of the postmortem material at the Gallinger Municipal Hospital, Washington, D. C., and the Charity Hospital, New Orleans, substantiated the observations of a number of Southern and Northern clinicians that coronary thrombosis secondary to coronary arteriosclerosis was rare in the negro race. Gross and microscopic examination of the aortas and coronary vessels obtained from negroes with arteriosclerosis, as compared to material from the white races, revealed deeper lying atheromatous plaques with greater subendothelial connective tissue proliferation, a decreased incidence of atheromatous ulcers and a greater involvement of the media by the atheromatous process, resulting in localized dilatation of the vessels which appeared to aid in the maintenance of adequate patency. These anatomical differences are offered as the chief explanation for the low incidence of coronary thrombosis in the negro. It is suggested that the atheromatous material acts as a mild irritant and that the subendothelial connective tissue reacts more vigorously in the negro than in the white, similar, perhaps, to the marked connective tissue reaction so commonly seen in the negro following skin and subcutaneous tissue injuries.

THE RELATION OF PERICARDIAL ADHESIONS TO CARDIAC HYPERTROPHY.  
R. M. Hosler (by invitation) and Francis Bayless, Cleveland, Ohio.

*Abstract.* It is generally considered that fibrous pericardial adhesions may cause cardiac hypertrophy. This report includes a study of 281 cases of adults with adherent pericardium found in a survey of 15,000 autopsies in the Institute of Pathology of Western Reserve University (1898-1936) and the Cleveland City Hospital (1915-1936). There were 182 males and 99 females.

The autopsy protocols were abstracted with verbatim descriptions and measurements of the hearts, the condition of the kidneys and lungs, the appearance of the arterioles, the weight and stature of the patients, the blood pressure, and other points of importance in assaying the relation between the weight of the heart and the type and distribution of the accompanying pericardial adhesions. In some instances the clinical record was also reviewed.

For comparison, normal heart weight figures for adult males and females were taken from the tables published by Roessle and Roulet (439 males and 115 females) and by Smith (534 males and 320 females). Curves were drawn to show these maximum and minimum normal variations for the different age periods in the two sexes. On these curves were plotted the weights for the 112 non-hypertrophic hearts found in the series (87 males and 25 females). There were no significant variations from the normal.

In the series there were also 95 males and 74 females with genuine cardiac hypertrophy. The mean heart weight for the males was 899.2 gm., for the females 548.9 gm. In every case there was an adequate explanation for the cardiac hypertrophy, other than pericardial adhesions, such as inflammatory heart disease, hypertensive heart disease, severe coronary arteriosclerosis and myocardial damage, extensive pulmonary disease accompanied by right heart hypertrophy, and various combinations of cardiac diseases.

The type and distribution of the pericardial adhesions were classified as (1) local, (2) subtotal, and (3) complete obliteration of the sac. The cases were also analyzed to show the situation and severity of extrapericardial, or pericardio-mediastinal, adhesions. The hypertrophic and non-hypertrophic hearts were then compared on this basis. There were 17.8 per cent hypertrophic hearts and 17.8 per cent non-hypertrophic hearts with Type I adhesions; 5.4 per cent hypertrophic hearts and 11.7 per cent non-hypertrophic hearts with Type II adhesions; and 76.8 per cent hypertrophic hearts and 71.5 per cent non-hypertrophic hearts with Type III adhesions. Calcified pericardium occurred with both hypertrophic and non-hypertrophic hearts.

*Summary:* The results of the study establish that cardiac hypertrophy is not related to pericardial adhesions. When the heart is hypertrophic an adequate explanation, other than pericardial adhesions, is always found.

#### *Discussion*

(Dr. Howard T. Karsner, Cleveland.) It is to be more strongly emphasized than the time limit of Dr. Bayless' presentation permitted that, in spite of the widespread teaching that pericardial adhesion causes cardiac hypertrophy, such teaching is no longer valid. Pericardial adhesion can now be removed from the list of causes of hypertrophy of the heart. Other studies of the condition have occasionally led to the same conclusion but they were not based on a number of cases as large as that covered in this report.

THE DEVELOPMENT OF AN ANTIVIRAL PRINCIPLE IN LYMPH NODES. Philip D. McMaster and (by invitation) John G. Kidd, New York City.

*Abstract.* Rabbits were inoculated intradermally in one ear with a standard amount of vaccine virus and in the other with typhoid bacterin. In half the instances the ears were amputated an hour later. After later intervals of from 2 hours to 15 days the cervical lymph nodes of both sides, inguinal nodes, bone marrow and spleen were removed and extracts of them were separately inoculated into normal rabbits (as was serum procured at the same time) to test for their content of virus. In addition, neutralization tests were set up to learn whether or not the materials possessed antiviral properties.

By the 2nd or 3rd day an increase in virus was demonstrable in the extracts of the nodes on the virus-injected side. A rapid decline in the amount took place thereafter and by the 7th to the 9th days the extracts failed to produce lesions. At no time did the serum or extracts from other nodes or organs give evidence of virus.

The neutralization tests showed an early appearance of neutralizing ability in the extracts of the nodes from the virus-injected side. By the 4th day 10 per cent extracts of these nodes inactivated fresh vaccine virus diluted to  $10^{-5}$  and  $10^{-6}$  to a greater degree than did whole serum. Serum of the same animal, similarly diluted, possessed no neutralizing power, nor did the extracts of inflamed cervical lymph nodes from the other side, nor the extracts of spleen or bone marrow or of either ear. Virus was found only on the virus-injected side, in the ears and cervical lymph nodes. The negative findings in the inflamed nodes on the side injected with typhoid bacterin served to control the possibility that a concentration of antiviral bodies formed elsewhere in the body and circulating in the blood had taken place in the nodes of the virus-injected side.

Experiments were done to test whether a neutralizing principle might be formed in the tissue of the injected ear, and reach the nodes by drainage. Extracts of the tissues of both ears as well as of the cervical nodes of both sides 4 days after inoculation were separately filtered through Seitz pads and tested. The filtration separated virus from antiviral principle in the extract of the cervical nodes from the virus-injected side, in which both were present together, only the antiviral properties passing the filters. The ear extract totally lacked antiviral power and so too did that of the glands on the control side, whereas that of the glands on the injected side possessed it. Only virus was present in the ear extract and only in that from the virus-injected side.

The results from day to day with serum and extracts of organs other than the lymph glands from the virus-injected side indicate that the latter were a major source of the neutralizing power of the serum.

### *Discussion*

(Dr. Paul R. Cannon, Chicago.) I have been particularly interested in this paper because for a number of years we have been interested in the problem of the local formation of antibodies. Our greatest difficulty has been in demonstrating antibodies before their appearance in the blood serum. I should like to ask Dr. McMaster what was the earliest time period at which antiviral substances were detected in the lymph nodes. It seems to me that the evidence here submitted is more conclusive than in most of the experiments that have been re-

ported for bacterial antigens and that these experiments are quite convincing for the demonstration of the local formation of antibodies.

(Dr. Jules Freund, New York City.) Dr. Rich, I believe, made some very interesting observations on the effect of immunization on lymphocytes. I think your study on the local formation of antibodies in lymph nodes offers a unique opportunity to investigate the possible rôle of lymphocytes in antibody formation. Were histological studies made which might reveal morphological changes in lymphocytes? I may add to this a statement as to a local formation of antibodies at the site of the injection of the skin. I wish to say that in our studies on immunization with heat-killed typhoid bacilli no local antibody formation was found in the skin at the site of injection. I am mentioning this, for there are statements in the literature that there is such a local antibody formation in the skin.

(Dr. Arthur W. Wright, Albany.) I should like to ask Dr. McMaster if by any chance he tried neutralization experiments using lymph node extracts from animals that had not been inoculated with virus but in which hyperplasia and hypertrophy of the lymph nodes had been produced by other means, in other words — extracts of hyperplastic lymph nodes of animals that had not had virus at all.

(Dr. McMaster, closing.) In reply to Dr. Cannon's question, we have not been able to find antibodies in the lymph node extracts before they appeared in the serum. We have always found them present in both, and all I can say is that they are stronger in the lymph node extracts than in the serum a few days after the animal has been injected with virus in one ear — for example, on the 4th day. By the 7th day they are as strong or much stronger in the serum than in the node extract. On the 3rd day we found some degree of neutralization by the lymph node extracts, but I do not believe that neutralization tests are good enough to distinguish between the small amount of antiviral principle found in the node extracts and that found in the serum at that time.

In reply to Dr. Freund, we have not made histological studies as yet. We have saved our sections, but so far I have nothing to report. We have not worked with the skin at all, except as it entered into the filtrates of ear extracts, which included the skin and all other tissues above the cartilage, and, if there were vaccinia lesions present, it included those too and any other inflammatory material from the ear. We worked with the extracts and filtrates of all these tissues, not with skin alone.

Concerning the question of Dr. Wright's about the possible presence of neutralizing principles in lymph nodes of animals suffering from infections other than vaccinia, we have used for controls those rabbits in which we injected typhoid vaccine in the other ear. Before we began these experiments we obtained lymph nodes from a series of animals and, by mixing extracts of these nodes with known amounts of virus, tested the effect of the extracts on the ability of the virus to produce lesions. In these preliminary experiments we also used extracts of enlarged and inflamed lymph nodes from some rabbits which had been injected in the ears with typhoid vaccine. All gave negative findings in neutralization tests.

THE MORPHOLOGY AND DISTRIBUTION OF THE INCLUSION BODIES OF THE SUBMAXILLARY GLAND VIRUS IN ADULT AND FETAL GUINEA PIGS. Floyd S. Markham (by invitation), Columbus, Ohio.

*Abstract.* Evidence based on a study of both fresh and fixed tissues suggests that the inclusions associated with the submaxillary gland virus of guinea pigs consist of a chromophile matrix in which are embedded corpuscles resembling those seen in certain other virus inclusions.

These corpuscles may be stained in Zenker-fixed tissues by Harris' hematoxylin differentiated in a half saturated solution of picric acid.

In tissues prepared by Gersh's modification of the Altmann technique, both the intranuclear and cytoplasmic inclusions give a positive reaction to the Feulgen stain for thymonucleic acid.

The inclusions in intracerebrally inoculated young guinea pigs are confined to the cells of the meningeal exudate, and inclusion-laden cells are not found in the circulating blood. Intracerebral inoculation of virus emulsions into the guinea pig fetus *in utero* is frequently followed by invasion of the blood stream by inclusion-laden mononuclear cells and the appearance of foci in most of the extra-cranial tissues, especially the placental mesenchyme.

*Discussion*

(Dr. S. Burt Wolbach, Boston.) This paper is personally very interesting because we, in Boston, encounter identical inclusions so frequently in the salivary glands and other organs of infants, newborn and stillborn. Perhaps you will recall that many years ago Dr. Allan Smith of Philadelphia regarded these inclusions as protozoan in character and submitted the preparations to Hertwig who pronounced them as cells, not of mammalian origin. Another interesting thing is that they are found so frequently in rodents. I should like to ask Dr. Markham the nature of the material which he used for inoculation — was it cell debris or filtrates? You may recall that Dr. Farber and I made a very careful study in the endeavor to correlate the presence of these inclusions with any one type of disease, but we found them distributed regardless of the nature of the illness exhibited or the cause of death. Needless to say, our opinion was strongly against these inclusions being the cause of pertussis, a theory which has been advanced.

(Dr. Markham.) In answer to your question about the nature of the inoculum, both types of inoculum have been used, that is, filtrates of emulsions of submaxillary gland and simple sedimented emulsions.

(Dr. Wolbach.) You did not employ human material?

(Dr. Markham, closing.) No, so far as the reports go I know of only one other instance, I believe that of Kuttner, who has reported attempts to infect rodents with emulsions of human material.

ON THE PATHOGENESIS AND IMMUNOLOGY OF POLIOMYELITIS. N. Paul Hudson and (by invitation) Edwin H. Lennette and Francis B. Gordon, Columbus, Ohio.

*Abstract.* Experimental evidence is presented to indicate that the olfactory nerve endings in the nasal mucosa are the portal of entry of the poliomyelitis virus. Five monkeys with sectioned olfactory tracts were not infected by intranasal virus, and later they did not show symptoms of poliomyelitis after intra-



venous inoculation of virus lethal to four of five controls. The possibility of infection here resulting from intravascular virus being exuded onto the nasal mucosa and thence by the olfactory tracts is supported by our finding the virus in nasopharyngeal washings of other monkeys after intravenous injections. No evidence of infection was found in four monkeys given huge and repeated amounts of virus-cord into isolated intestinal loops with external openings. Another possible route of infection is by the blood stream to the central nervous system. Against this as a natural route is the fact that only very large intravascular injections are infective, and furthermore the existence of a blood-CNS barrier was demonstrated by experimental infections resulting from small intravenous doses of virus at the same time that injections of sterile starch were made into the brain. From these experiments it appears more clear that the virus of poliomyelitis under natural conditions enters the central nervous system through the exposed hairs of the unmyelinated nerve fibers of the olfactory tracts.

Evidence furnished by other workers shows the intraneural migration of the virus to the cord. We propose that the same occurs even without signs of infection and that there is an overflow of virus into extraneural tissue with the resulting formation of antibodies. In support of this view, the virus has been recovered by us from perfused spleens of two monkeys during the acute attack after cerebral inoculation. We consider on the basis of our experimental studies that the neutralization test in man and monkeys is a specific antigen-antibody reaction, and under natural conditions an evidence of extraneural stimulation of antibody-forming tissues after neural migration of virus. Under conditions of artificial immunization in the monkey, specific antibodies may be induced, but measures to establish an effective immunity against experimental infection have been found necessarily so extreme that they are correspondingly unsafe for human application. Furthermore, the presence of serum antibodies, as we have experimentally determined, is not a certain indication of effective resistance to virus administered by the natural intranasal route.

### Discussion

(Dr. Jules Freund, New York City.) I think that in judging the possible rôle of antibodies in the resistance to poliomyelitis it is desirable to consider not only the presence or absence of the neutralizing antibody in the blood, but also the titers of the serums. It is possible that in poliomyelitis the antibody content of the central nervous system is independent of that of the blood, but with agglutinins against typhoid bacilli there is a numerical relation between the antibody content of the blood serum and that of the spinal cord, the ratio being 100 to 0.8 (*J. Exper. Med.*, 1930, 51, 889).

(Dr. Hudson, closing.) In reply to Dr. Freund, we are not able to answer Dr. Freund's question at the moment. The work on the quantitative side is being done. We feel, however, that the neutralization test is a rather crude test and, whereas some information may be given by the titration of antibodies, the variations that have occurred are too frequent for us to draw any fine and quantitative line. We realize that we can immunize against poliomyelitis experimentally, and presumably what Dr. Freund has indicated follows. However, what we are trying to point out here is that when we use a method which is close to the fatal line, a severe method, in the presence of antibodies, still such a condition is not effective against the intranasal administration of the virus. This point is well taken and is being pursued.

ON THE PROBABLE NATURE OF THE INFECTIOUS AGENT OF TRACHOMA. L. A. Julianelle, St. Louis, Mo.

*Abstract.* The studies published from this laboratory have already established trachoma as an infectious disease, transmissible to monkeys and apes. Independent of faulty or deficient diet, it was shown that neither bacteria cultivated from the conjunctival sac, nor filtrates of infected tissues obtained by filtration with Berkefeld V filters can be assumed as logical factors in the infection. In developing further the concept of infectivity of trachoma, it was found that active tissues retain their original potency after passage through rabbit or guinea pig testicle. Tissues originally non-infectious do not acquire infective capacity by testicular passage. By this technic, the infectious agent apparently does not multiply but is merely preserved over a period of 2 weeks or more, which suffices for purification of the original material from the extraneous bacteria usually present in trachomatous eyes. Sections and impression smears of active testicles fail to show any definite histological change, and no structures, including the so-called initial and elementary bodies of the human, have ever been observed. It appears, therefore, that the infectious agent of trachoma is a virus.

Attempts to cultivate the virus from human, monkey and rabbit tissues have thus far been unsuccessful. Inoculation of active material in minced chick embryo, in fertile eggs, and in plasma clots containing epithelial cells has been tried repeatedly without success. Inoculums of conjunctival cells direct from infectious patients in their own plasma clots have yielded distinct growth of epithelial cells, but the virus not only did not grow but even failed to survive the conditions of tissue culture. Experiments are still under study on cultivation of the virus, so that it is not desirable to make any conclusions.

Recognizing the difficulty of generalization, since the variation in infectivity of tissues and susceptibility of animals is great, it seems fair to offer the following characteristics of the trachoma virus. It is inactivated by heating at 45 to 50° C. within 15 minutes; is rendered non-infectious by the action of bile which causes lysis of epithelial cells and inclusions; does not survive exposure to dilutions of 1:100,000 gentian violet, 0.25 per cent phenol, 2 per cent silver nitrate, 4 per cent cocaine, and in about half the tissues tested, even 2 per cent cocaine. Preservation of the virus in 50 per cent glycerine, still under study, indicates that glycerine does not maintain the virus any longer than storage without preservative at icebox temperature. Ordinary preservation at this temperature varies with individual tissues up to a week or more; at room temperature the virus may survive up to 24 hours; at body temperature survival is a matter of hours only. While singly none of the characteristics depicted helps to identify the agent, collectively they suggest, as brought out by the testicular experiment, that the infectious agent of trachoma is a virus. The part played by the epithelial cell inclusion will have to await future investigation for possible clarification.

*Discussion*

(Dr. Edward C. Rosenow, Rochester, Minn.) I should like to ask whether the material inoculated into the rabbits from which the virus was derived does or does not contain bacteria, especially *B. granulosis*, as described by Noguchi.

(Dr. Julianelle, closing.) The material which comes from the eyes in trachoma is always contaminated with extraneous bacteria, and those bacteria are inoculated with the tissues in the rabbits. The testicular tissue after a couple of weeks

is bacteriologically sterile. In some instances *B. granulosis* described by Noguchi was present, but this organism does not cause any reaction in monkeys, so there is no particular point in discussing it.

THE PATHOLOGY OF THREE CASES OF GLYCOGEN STORAGE DISEASE ILLUSTRATING SOME UNUSUAL FEATURES. F. W. Wigglesworth (by invitation), Montreal, Canada.

*Abstract.* The 3 cases to be presented were observed within a period of 1 year at the Children's Memorial Hospital, Montreal.

*Case 1.* This child, a male, aged 3 years, presented the typical picture of von Gierke's disease, both clinically and biochemically. Biopsy of the liver was done and estimation of the glycogen showed the liver to contain 18 per cent. Sections demonstrated an early portal cirrhosis with greatly swollen liver cells packed with glycogen. The child died 3 months later of pneumonia.

At autopsy the liver weighed 2155 gm. Chemical analysis showed the liver to contain 11 per cent glycogen and the sections revealed a corresponding decrease as compared to the biopsy specimen. The polymorphonuclears in the lung were laden with glycogen, while the other organs contained only traces of it.

*Case 2.* A male infant, aged 2 months, a younger brother of the patient in Case 1, died of meningococcal meningitis 24 hours after admission to the hospital. Sections revealed a moderate amount of glycogen in the liver cells, although autopsy was performed 10 hours after death. This case points to a definite familial tendency of the disease.

*Case 3.* A female infant, aged 11 months, who clinically did not suggest glycogen storage disease, was diagnosed as suffering from cirrhosis of the liver. The blood sugar was 42 mg. per cent — a finding that might have led to the proper diagnosis. Autopsy showed a liver weighing twice the normal weight with gross changes suggesting cirrhosis. Histologically there was a well developed portal cirrhosis with large liver cells containing glycogen, although the tissue had been fixed in watery fluids. The spleen showed moderate amounts of glycogen in the reticular cells.

The findings in these 3 cases point to the following conclusions:

1. That the disease may lead to cirrhosis.
2. That there is a familial tendency.
3. That the condition is present from an early age and before signs or symptoms can be noted.
4. That the reticular cells of the spleen may take up glycogen.
5. That possibly the glycogen is mobilized during an acute disease such as pneumonia.

### Discussion

(Dr. Edwin F. Hirsch, Chicago.) Did any of these patients have symptoms of diabetes? The clinicians of St. Luke's Hospital, Chicago, have a boy 17 years of age, apparently afflicted with this disease, but also showing symptoms of diabetes. The results of the adrenalin and glucose tolerance tests were those of a patient with diabetes. A small piece of liver tissue removed for examination showed the changes that occur with glycogen storage.

(Dr. David Perla, New York City.) Did you test for glycogen in organs other than the liver? It is a very interesting thing that in experimental infections of many types the normal storage of glycogen very rapidly diminishes, and in these

animals it is almost impossible to determine the presence of any glycogen at all in the liver, whereas the normal animal may contain large amounts. Have you any idea as to the nature of the disturbance in this disease?

(Dr. H. Edward MacMahon, Boston.) Dr. Wiglesworth has pointed out that the polymorphonuclear leukocytes in the inflammatory exudate in the lungs of one of his patients showing this interesting disease contained glycogen. In making routine studies of pneumonic exudates it is quite common to find a moderate amount of glycogen in these cells. I should be interested in knowing if the amount of glycogen in the polymorphonuclear leukocytes was increased in this disease, and if from a diagnostic standpoint the presence of glycogen in the leukocytes of the circulating blood is of value.

(Dr. Wiglesworth, closing.) I may say that in the first case which I showed, and which we followed for  $1\frac{1}{2}$  years before death, there was no evidence of diabetes and no sugar in the urine at any time. The other cases were followed for only brief periods, but they had no sugar in the urine.

As to the presence of glycogen in the other organs, the muscle at biopsy showed some. I cannot say whether there was an excess or not. The rest of the organs at autopsy showed no glycogen in any amount. Chemical analysis of the brain, spleen and kidney showed traces of glycogen.

As to the nature of this condition, I am afraid I do not know. The condition is thought to be a continuation of the fetal metabolism. Apparently in the fetus the glycogen is extremely stable, just as it is in this condition.

Concerning the exudate in the lungs, polymorphonuclears were examined for glycogen during life and there was no glycogen in them at that time, when the child was in perfect health; but, at autopsy, sections of the various organs showed polymorphonuclears containing glycogen in the blood vessels.

**PATHOLOGY OF THE URACHUS.** James E. Davis and (by invitation) George Hammond and L. Yglesias, Detroit and Ann Arbor, Mich.

*Abstract.* The pathology of the urachus is closely linked with and influenced by the developmental history of the urinary bladder and the midportion of the anterior abdominal wall. The completed transition of the cloaca and allantois, with the regression of the umbilical arteries and apical part of the bladder wall to ligamentous supports, is also of great importance.

Obliteration of the urachal canal by atrophy of its epithelial lining depends on elongation of the body trunk, thereby separating widely the umbilicus and the bladder apex, and also on lateral tension of the two strongly retrogressing umbilical arteries. These, together with abandoned function, should normally convert urachal structures to a ligamentous cord with effectual prevention of fractional function of its epithelial cells. The degree of incompleteness in the foregoing changes determines urachal pathology.

The literature on the urachus should be extended as an enrichment to our knowledge in urology.

Sixty dissections of the urachus in fetuses and newborn infants have been made by the authors and interesting anatomical observations have been recorded pertaining to:

1. Complete covering of the urachus by peritoneum in all cases.
2. Closure of the bladder end of the urachus by Wutz's ligament in all normal formations.
3. The obliterated umbilical arteries and body growth elongation which cause striking variations in the ultimate intactness of the urachus even at birth.

4. A firm distinct fibrous sheath, separable from the peritoneum and anterior to it, which is found between the umbilical arteries and folded about them.

5. Variations in the urachus which were noteworthy in umbilical hernias and ectopias.

Four new cases of carcinoma are reported, all of which appear adequately proved, with 1 more, lacking in diagnostic data.

Three additional unproved tumor cases are cited.

Three differential diagnostic cases are mentioned.

### Discussion

(Dr. Theodore J. Curphey, Westbury, N. Y.) I should like to ask whether Dr. Davis draws any distinction between the fibroadenomas, or adenomyomas, and endometriosis of the umbilicus, which I think Dr. Weller described some time ago. Are they different lesions?

(Dr. Davis, closing.) In Begg's contribution to the tumors of the urachus (*Brit. J. Surg.*, 1931, 18, 422) displacements of the epithelial lining into the surrounding connective tissues are well pictured. This epithelium is of transitional type with here and there goblet cells, thus differing from the endometrial epithelial pattern. In the adenomyomatous formations or in endometriosis the taller endometrial cells are surrounded by extravasations of red blood cells at each menstrual period. In all male subjects endometriosis and adenomyosis would at once be excluded. The lesions of urachal origin are different lesions.

RETINAL TUMORS IN TUBEROUS SCLEROSIS. B. Earle Clarke, Providence, R. I.

*Abstract.* Involvement of the retina in cases of tuberous sclerosis escaped notice until 1921 when Van der Hoeve (*Arch. f. Ophth.*, 1921, 125, 880), a Dutch ophthalmologist, published a clinical report describing 6 cases in which tumors of the retina were observed through the ophthalmoscope. Since then a number of clinical reports have appeared, indicating that such retinal tumors are not uncommon and that disturbance of vision and the ophthalmoscopic examination may give the first clue to the disease.

Two years later (1923), Van der Hoeve (*Arch. f. Ophth.*, 1923, 111, 1) published a pathological study of the retinal tumors from 2 of his 6 cases. From 1 he had only one eye which was enucleated because of a suspicion of malignant change. In this eye there was a large tumor of the optic disc, which he regarded as the primary growth, and several smaller secondary tumors scattered over the retina. In the other case, both eyes were removed at autopsy. There were no tumors of the papillae but several retinal tumors in each eye. The histology in both cases was essentially alike. He described the tumors as being made up of nerve fibers and a peculiar kind of cell. The fibers, he believed, came from the nerve fiber layer and extended through holes in the membrana limitans interna into the overlying tumor. The cells are described as having much cytoplasm which, in places, fused with that of neighboring cells to form a syncytium. The nucleus was usually large with a prominent nucleolus. In the papillary tumor there was a large incrustation. In all the tumors there were spaces without any special lining filled with blood and serum. Blood vessels were sparse. The tumors appeared to originate in the nerve fiber layer and frequently extended to involve the ganglion cell layer, but seldom any others. On the surface of the large tumor of the optic disc were button-like projections which, he believed, became pinched off to float in the humor and then became implanted

elsewhere on the retina. Indeed, he actually observed this occur through the ophthalmoscope.

I have been able to find only three additional pathological studies of these tumors — all in German. In 1925 Schob (*Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1925, 95, 588) published his findings in the eyes of a 6 year old child who died of tuberous sclerosis. There were no tumors of the disc. Grossly, small whitish, slightly raised areas could be seen on both retinas. Microscopically some were flat and some sharply raised. He described buds and daughter buds with constricted necks sitting on the surface of the larger tumors. In his case the tumors were limited to the nerve fiber layer. He, too, described fibers but concluded that they were definitely glial fibers and not nerve fibers. He also described a syncytium and giant cells with two or three nuclei. The nuclei were large and vesicular with little chromatin and with one to three nucleoli. They were oval, long, curved and sometimes dumb-bell shaped.

In 1930 Feriz (*Virchows Arch. f. path. Anat.*, 1930, 278, 690) reported a histological study of a solitary tumor of the left eye which did not involve the disc. This tumor involved the nerve fiber layer and the ganglion cell layer. He described large "spider and star-shaped cells." The cytoplasm stained strongly eosinophilic. In places the cell walls were indistinct, suggesting a syncytium. Many cells contained vacuoles. The nuclei were round to three cornered. There were bands and whorls of fibers which he considered "neuroglia-like."

The third such study was by Kuchenmeister (Report of Proceedings of 10th Day of Bavarian Ophthalmological Congress, München, *Ztschr. f. Augenhe.*, 1935, 88, 158) in 1935. Both eyes were involved. The optic discs were not involved. Unlike the previous reports, all layers were invaded. In places there was calcification and in the chorioid some bone formation. The cells varied in size and shape. There were "new formed glial fibers."

On March 17, 1935, a white male, 20 years of age, was admitted to the Rhode Island Hospital with pneumonia. A history of epilepsy was obtained and it was later learned that he had been under treatment at the skin out-patient department for adenoma sebaceum of the face. There was failing vision of the right eye and, on ophthalmoscopic examination, a tumor 3 mm. in diameter was seen over the optic disc.

At postmortem examination typical lesions of tuberous sclerosis were found, including multiple brain tumors, rhabdomyoma of the heart, lipofibroma of the kidney and adenoma sebaceum of the Pringle type. In the right eye was a single, raised, whitish tumor, 3 mm. in diameter, which covered the upper and inner two-thirds of the disc.

Histological preparations show the tumor overlying the optic disc and, as in Kuchenmeister's case, involving all layers of the retina. It also invades a portion of the optic nerve. In the central portion of the tumor is a large, irregular mass of ossification and about this are calcium-containing concretions. The surface of the tumor is smooth; there are no buds, as were described by Van der Hoeve and Schob. At the periphery of the tumor a thin layer of tumor cells extends out over the surface of the external limiting membrane.

The cells vary greatly in size and shape. Some are almost round. Others are markedly angulated. The cell boundaries are often indistinct, suggesting a syncytium. Many cells are elongated and the cytoplasm is drawn out in a thick process. It occurs to us that this is an attempt to form rod and cone cells.

The nuclei are round or oval and vesicular. A single nucleolus is frequently prominent. Multinucleated cells are seen having from two to five nuclei.

Phosphotungstic acid hematoxylin preparations failed to show any production of glial fibrils.

The cell type is not definitely determined. Van der Hoeve suggests that these large cells are descendants of the first anlage of the retina — a kind of neurocyte, "glia-neurocyte," which has not differentiated into glia or ganglion cells. Believing that they arise from embryonic cell rests, he proposes that they be called "phakomata" from the Greek word "phakos" meaning "mother-spot." Combining these two words he arrives at the term "neurocytophakomata retinae" or "papillae."

Schob concludes that these tumors are closely related to other gliomatous tumors and that they take origin from the nerve fiber layer which normally contains glial cells. He proposes no definite name.

Feriz considers these tumors very much like those seen in the brain and believes that they are made up of atypical neuroglia-like tissue.

It seems evident that these tumors must arise from some early cell of the embryonic retina which still is potentially able to differentiate in more than one direction. It appears definite that glial fibers were present in the cases of Schob, Feriz and Kuchenmeister. None were present in Van der Hoeve's case, nor could I demonstrate any in mine. Van der Hoeve describes nerve fibers which were not seen in any other cases. In my case there is at least a suggestion of differentiation into rod and cone cells. This is not described by any other writer.

Such embryologically derived terms as "retinoblastoma," "retinal neuro-epithelioma," and so on, have already been used to designate quite different tumors. In view of our incomplete knowledge at present it is perhaps wisest to speak of them only as the retinal tumors of tuberous sclerosis.

#### PROTECTION AGAINST TUBERCULOSIS BY EXPERIMENTALLY PRODUCED GHON TUBERCLES. Benjamin J. Clawson, Minneapolis, Minn.

*Abstract.* Experiments were undertaken to determine whether from the standpoint of immunity the Ghon tubercle has a good or bad effect on the further pathogenesis of pulmonary tuberculosis.

Non-progressive Ghon tubercles were produced in the lungs of rabbits by three methods: (1) by injecting a few dried clumps of BCG intravenously; (2) by injecting 2 mg. of living BCG into the lungs with a long needle through the trachea; and (3) by injecting the same amount into the lung through the thoracic wall and pleura. All such animals became allergic, as shown by a positive Mantoux test.

These rabbits with a series of control rabbits were later inoculated subcutaneously with 0.01 mg. of a virulent strain of bovine tubercle bacillus (Ravenel). Forty-five days later all animals were killed. Those having the experimentally produced Ghon tubercle showed little or no tuberculosis, while the control animals had extensive tuberculosis.

#### Discussion

(Dr. David Perla, New York City.) I should like to know what the regional lymph nodes showed.

(Dr. Jules Freund, New York City.) I should like to ask if titration of antibodies and tuberculin tests were made, and if so, the results. Was any one of the three methods of injecting BCG superior to the others?

(Dr. Esmond R. Long, Philadelphia.) It may be interesting to recall experiments by Dr. Bloch of the University of Chicago on the production of similar solitary nodules. He mixed tubercle bacilli with lipiodol, and the oil seemed to hold the bacilli in a single mass. He was able to show the lesions roentgenographically and subsequently watch their development. I think the lesions he produced were a good deal like those Dr. Clawson showed, although, as I recall, they were not as sharply localized on the pleural surface. It is interesting to know what the lymph nodes showed in these cases. The Ghon tubercles are the characteristic lesion of healing childhood tuberculosis, and one of the typical features of the childhood type is enlargement of the lymph nodes with caseation, more or less in contrast to what we see in the adult type.

(Dr. Theodore J. Curphey, Westbury, N. Y.) Are these experimental tubercles calcified, even after the 45 day period?

(Dr. Clawson, closing.) The lymph nodes with these localized lesions were not involved. I used living BCG on purpose in order to get a lesion which was non-progressive. With such lesions there was no lymph node involvement. When I used another strain, a bovine strain of low virulence, the lymph nodes did become involved. Allergy was present in all. The rabbits did show a fair degree of antibody content, as indicated by agglutinins.

In reply to Dr. Freund's question as to which of the three methods is better, I would say all three methods from the standpoint of protection appeared to be about of equal value. The last method from the standpoint of producing the localized lesion was by far the best.

In reply to Dr. Curphey, the nodules did become calcified and could be seen by the X-ray. Grossly they had not gone long enough to show a calcified nodule, but microscopically calcium was present.

BRONCHIOGENIC DISTRIBUTION OF PARTICULATE MATTER: ITS SITE OF PREDILECTION AND THE MECHANISM OF TRANSFER. Herbert S. Reichle, Cleveland, Ohio.

*Abstract.* Observations on human lungs injected with formalin and sectioned in the coronal plane show that the site of predilection in bronchiogenic distribution of particulate matter is a transverse band area involving, on the right, the lower one-third of the upper lobe and the apex of the lower lobe; on the left, the middle one-third of the upper lobe and apex of the lower lobe. The same site is involved when rabbits and guinea pigs are injected intratracheally with small amounts of India ink. The bronchi supplying these sites on the right are the two caudal branches of the bronchus eparterialis and the first dorsal branch of the main stem bronchus hyparterialis; on the left, branches of the bronchus ventralis I (Aeby) and the first dorsal branch of the main stem bronchus hyparterialis. Approximately similar results are obtained with the animal in supine, lateral or dorsal position. Gravity, therefore, is not a sufficient cause for the production of these sites of predilection. An additional factor — of preponderant importance in the dorsal position — is the structure of the tracheobronchial tree. The dorsal aspect of the trachea is not flat; in the well preserved specimen it shows lateral grooves separated by a membrane which is thrown into high longitudinal folds. These grooves are continued into the main bronchus both by the folds and by a projecting ledge of cartilage. If the system is not flooded, this structure insures a *marginal flow* and particulate matter suspended in liquid is shunted into the lateral and dorsal branches of the main stem bronchi.



A different mechanism results when particulate matter is suspended in air. It then proceeds by *axial flow*, which is not dependent on the structure of the bronchial wall. The distribution to the lung is governed solely by the breathing space; it is therefore roughly uniform. This mechanism has been demonstrated by subjecting guinea pigs to inhalation of air charged with animal charcoal.

**TYPICAL TUBERCULOUS COMPLEXES (RANKE) IN POSTPRIMARY TUBERCULOSIS OF THE LUNGS.** Kornel L. Terplan, Buffalo, N. Y.

**Abstract.** In a paper delivered 2 years ago at the meeting of this Association in Toronto, we mentioned a few instances of certain types of tuberculous reinfection which did not support the usual conception of localization; size and spread of the so-called reinfection. Incidental findings of focal tuberculous lesions in the lungs and in lymph nodes draining the site of these foci in individuals who did not die from tuberculosis resembled very closely lesions seen heretofore only in primary tuberculosis. In the past 2 years our systematic studies on tuberculous lesions in the lungs were continued, and more cases of this type were discovered. Restricted focal lesions, which alone were examined in this series, are a more suitable material for pathogenetic analysis than extensive chronic tuberculous lesions in patients who have died from pulmonary tuberculosis.

The anatomical findings in 6 cases showing typical Ranke complexes of post-primary tuberculosis are presented. An exogenous tuberculous reinfection brought about the formation of a second Ranke complex. In all these cases it was felt on gross inspection that we were dealing with typical primary tuberculous complexes of relatively recent age. However, in addition, roentgen examination of the lungs and all the bronchomediastinal lymph nodes revealed in 3 cases entirely calcified and partly ossified complexes. In 3 other cases additional calcified and partly ossified Ghon foci were found without gross or microscopic evidence of calcified lesions in the regional lymph nodes. The histological study of all the lesions observed at gross dissection and after roentgen examination proved that in each case there were present two tuberculous complexes of decidedly different ages. In other words, the cheesy complexes seen at postmortem appeared as tuberculous complexes of a later infection, although their anatomical-histological picture did not differ from a typical tuberculous complex of primary tuberculosis. In 4 cases the tuberculous complexes were in different lungs, in 2 cases in different lobes of the same lung, but in areas with a separate lymph drainage, like the upper third of the upper lobe and the base of the lower lobe. Histological examination showed the Ghon foci and the lymph node changes of first infection in an entirely healed, calcified or ossified stage. The complexes of postprimary infection appeared in a still active tuberculous stage; all of them were caseous. In most of these, however, there was a distinct tendency to progressive encapsulation with beginning chalky changes and very slight calcification in the center. It seems probable, therefore, that these complexes of postprimary tuberculosis also tend to become gradually calcified. The older they are, the more they may resemble in their size and structure the tuberculous complexes of first infection. If, therefore, in an adult two or more calcified tuberculous complexes are found in different lobes or in different lungs, it does not necessarily mean that we are dealing with multiple primary complexes or foci. The findings reported here show rather that postprimary tuberculous complexes cannot be ruled out.

In not one of these cases was anything known of serological reactions pointing to an active tuberculous process. Further, results of tuberculin tests were not

available, as all these individuals died from different causes, such as peritonitis, mesenteric thrombosis, acute coronary disease, and so on. It is most likely that the state of immunity acquired by the first tuberculous infection had been lost entirely, so that a second tuberculous infection brought about a lesion identical with that of a primary complex.

In the literature there are only two notes that refer to somewhat similar findings; one by Schuermann, who saw a recent cheesy complex in the intestinal tract in addition to an older calcified complex in the lung, and a similar observation by Anders. In both cases, however, the anatomical picture was complicated by more active tuberculous lesions in other organs.

In the 6 cases presented here we are dealing with almost entirely closed complexes. In only 2 of these were a few hematogenous fibrous tubercles which apparently belonged to the second infection found in the spleen and liver. These all showed a distinct tendency toward healing.

Although we have examined more than 200 cases systematically in the last 4 years, and although we have encountered tuberculous complexes of postprimary infection in at least 10 instances, we feel that the material is too small to be used for estimates as to the relative frequency of this type of reinfection.

### *Discussion*

(Dr. Herbert S. Reichle, Cleveland.) There have appeared in the past few years, and I am thinking particularly of an article recently published in the *American Review of Tuberculosis*, a number of reports of careful studies of cases in which the tuberculin tests after being positive became negative. When I use the word negative, I mean cases in which the tuberculin dilutions had been run down to 1:10 with the use of all the necessary precautions. Since we find cases in which the tuberculin test again becomes positive after the individuals are subjected to a source of possible reinfection, it would be of great interest, as Dr. Terplan has indicated, to find out whether these individuals belonged to this group in which there has been a biological healing of the Ghon complex and whether individuals are susceptible to a second Ghon complex.

(Dr. Jules Freund, New York City.) I should like to say something that is confirmatory of Dr. Terplan's paper. I have had an opportunity to test 500 nurses in the last 4 years, and also a large number of medical students from year to year. I have found a few negative reactions in people who have definite evidence of a former infection. Tests were made with 1 mg. tuberculin at yearly intervals. Sometimes these persons were positive at the first test, and a year later were negative. Some were tested for 2 or 3 years and were constantly negative. I cannot give the exact figures at present.

(Dr. Terplan, closing.) With regard to Dr. Reichle's discussion, I should like to say that, in a series of children examined, I found a number of Ghon foci which had completely healed. Although complete serial sections were made, no signs of tuberculosis were found in the regional lymph nodes which drained the site of those foci. In only 2 of these were results of the tuberculin reaction available; in these the reaction had been negative in the year previous to death. One child died from a brain abscess and the other from uremia. It is not improbable that repeated serial tests in older children and young adults would show a considerable number of negative tuberculin tests which at previous examinations had been positive.

THE LEUKOCYTIC RESPONSE IN TUBERCULOUS RABBITS FOLLOWING THE ADMINISTRATION OF TUBERCULIN. William H. Feldman and (by invitation) Joseph Stasney, Rochester, Minn.

*Abstract.* In an attempt to obtain experimentally a leukocytic response comparable to the so-called "leukemoid" reaction described as occasionally associated with tuberculosis of human beings, a group of ten tuberculous rabbits was studied. The hematological observations were restricted to the quantitative and qualitative characteristics of the white blood cells. The results indicate quite definitely that tuberculin given to sensitized rabbits provokes an elevation of the leukocytic count which is often of striking proportions. The increase is predominantly granulocytic in character and there occurs a marked shift to the left. The definite hyperplasia of the bone marrow, mitosis of the immature myeloid cells of the peripheral blood and other significant changes suggest a condition similar to the "leukemoid" reaction. The monocytes participate in the leukocytosis rather insignificantly, while the number of lymphocytes shows a definite tendency to diminish during the reaction that follows the administration of tuberculin. Tuberculin given to non-tuberculous rabbits has no significant effect on the white cell count.

*Discussion*

(Dr. David Perla, New York City.) I should like to know what happened to the normal rabbit. I did not notice whether you mentioned it. Also, have you any data on the quantity of nuclear protein in the OT you used, because it has been shown that nuclear protein stimulates granulocytic formation in the bone marrow?

(Dr. Theodore J. Curphey, Westbury, N. Y.) I should like to comment on Dr. Feldman's work in connection with some similar experiments that Dr. Russell and I performed in human beings a few years ago in attempting to study the changes in the bone marrow response in tuberculous patients following an intracutaneous injection of old tuberculin. The idea was to develop a functional hematopoietic test in this disease, similar to the glucose tolerance test in diabetes. We thought we could show a correlation between the extent of the shift to the left and the degree of involvement of the lung. For a while we thought the response was a specific one, but we were surprised to see that similar changes could be produced by the use of sterile milk in patients with the production of curves quite identical to those obtained by the use of OT. This, I think, will partly answer Dr. Perla's question.

(Dr. Kornel Terplan, Buffalo.) My interest in Dr. Feldman's experiments concerns primarily their application to human pathology. In certain types of chronic miliary tuberculosis bone marrow reactions of a leukemic or leukemoid nature have been observed. I remember one particular case the blood films of which were seen by the best authorities in hematology and diagnosed as leukemia. When the patient died, however, he showed only chronic hematogenous tuberculosis from which he had suffered for a half year. There were extensive lesions in the lungs, liver and spleen. The bone marrow did not show tubercles, and there were no leukemic changes in the organs mentioned.

(Dr. F. E. Chidester, Boston.) May I suggest that the use of milk may initiate the same type of reaction obtained with tuberculin and cause the very same effects on the glands of internal secretion, particularly those eliminating iodine, and that the relation of the thyroid to the problem of immunity has been ignored successfully, but not skillfully, since the days of Sajous?

(Dr. Feldman, closing.) In answer to Dr. Perla's question about the normal or the non-sensitized rabbits, these failed to give any change after the administration of OT. I cannot tell you as to the quantity of tuberculo-protein. We used tuberculin prepared by Parke & Davis & Co., and Dr. Long kindly sent us some PPD which we used on a small series. We did not have enough of his material for more, because it takes quite a lot of material with the doses used in this study. We do not feel this is an example of a specific reaction. We think other agents may elicit this sort of response, and we are at the present time investigating that angle of it.

I refuse to become engaged with the subject of Dr. Chidester's remarks — I do not know anything about it.

#### THE SIMILARITY BETWEEN THE REACTION TO SILICA AND TO TUBERCLE BACILLI.

Leroy U. Gardner, Saranac Lake, N. Y.

*Abstract.* The primary reaction to both silica and tubercle bacilli is phagocytosis by mononuclear leukocytes of the histiocytic type. The morphological, tinctorial and supravital staining reactions of the cells responding to these irritants are identical. In both silicosis and tuberculosis the histiocyte is modified to form epithelioid cells and giant cells of the Langhans type. Both irritants cause necrosis of tissue with the liberation of free fat and an exudation of leukocytes and serum. In each case the necrotic matter may undergo subsequent calcification. Both stimulate proliferation of histiocytes and fibroblasts that are laid down in the form of a compact nodule. About the periphery of each lesion is a more or less well defined zone of lymphocytes.

Differences between tubercle and the silicotic nodule are due to the nature of the irritants. Tubercle bacilli are alive and capable of indefinite multiplication but their rate of growth is more or less constant and is quite well balanced against that of the host's cells. As a consequence all *primary* tubercles are very similar in structure. As soon as excessive numbers of bacilli have formed, the equilibrium is disturbed, the tissues degenerate and the organisms spread into new areas. The character of the silicotic nodule varies with the number and size of the silica particles that have been concentrated in a focal area. Large numbers of very fine particles produce nodules with extensive central necrosis; smaller quantities of somewhat larger particles cause proliferation with little or no degeneration. Tubercles spread by direct extension and by metastasis through blood and lymph vessels and duct systems; silicotic nodules are enlarged gradually by peripheral expansion. Metastasis is generally limited to the regional lymph nodes. The fibrosis of silicosis is unique and is characterized by a peculiar hyaline swelling of the intercellular collagenous fibers. This is possibly a protective reaction that prevents diffusion of toxic material from the interior of the nodule.

The fact that a simple inorganic compound like  $\text{SiO}_2$  can set in motion a complicated series of cellular reactions comparable to those produced by a living organism made up of proteins, lipoids and carbohydrates is cause for wonder. It challenges the generally accepted views on the action of the tubercle bacillus. It suggests a search for a common physiochemical change produced in the tissues by the lipoids of the bacillus and by the molecule of silica. Both of these irritants are relatively insoluble but both theoretically excrete small amounts of toxic material over prolonged periods. Since the exact nature of the injury produced by each of these substances is still unknown it is profitable to correlate any relevant data.

*Discussion*

(Dr. Thomas H. Belt, Toronto.) Dr. Gardner's interesting work is of especial interest to those who have been working with silicosis, and I think it touches upon a problem which is of vital importance in connection with pneumoconiosis, namely the distinction between tuberculous lesions and the so-called "dust" lesions. As those pathologists who have examined the lungs from cases of silicosis well know, the majority of these people die with both active and healed tuberculosis in their lungs, and it is often an extremely difficult problem to distinguish which lesions are due to the silica or to the dust, and which are due to the tubercle infection. The chief difficulty arises in distinguishing the healed tuberculous nodule from the silicotic nodule, and in many cases I think it is impossible to make a satisfactory distinction between them. Dust particles will often be found incarcerated within the confines of a healed tuberculous lesion. One can find dust in appreciable quantities in both the healed and active tuberculous lesion; and in those cases of pneumoconiosis where death is due to extensive tuberculosis superimposed, one can see all stages of the tuberculous lesion, right from the earliest caseous or proliferative cellular lesion to the healed nodule. It is difficult indeed, as I have stated, to say definitely which are silicotic and which tuberculous nodules. I have often been led to wonder with other people, notably Kettle, and some of the other pathologists who have worked on silicosis, whether or not the majority of the silicotic nodules may not be really healed tubercles modified somewhat by the presence of the dust; but, one suspects in many cases it represents a lesion due to both the tubercle bacillus and the dust. I should like to ask Dr. Gardner about these lesions which he has produced in rabbits, the beautiful illustrations of which show giant cells and a very cellular reaction. Is there not some possibility that there may have been an element of infection in them, not necessarily tubercle infection? I should also like to know whether or not he has stained these lesions for bacteria and if he thinks they are due entirely to dust particles.

(Dr. Herbert S. Reichle, Cleveland.) In Cleveland we have exactly the same difficulty in distinguishing between pneumoconiosis and tuberculosis of the chronic fibroid type which Dr. Gardner has demonstrated so beautifully in his animals. I believe there are a fair number of cases in which, so far as one can ascertain, there is no tuberculosis. Certainly, so far as examination of the cavities can be made, they show nothing that is typical of an active exudative tuberculosis. Some of these patients have been examined for months in a sanatorium and their sputum, which is quite plentiful, never contained tubercle bacilli. In some cases this sputum has been put into animals and they have not succumbed to tuberculosis. I think it is therefore quite reasonable to suppose that pneumoconiosis can of itself give rise to serious conditions. I am thinking of one particular individual in whom there was a cavity of such a size as to erode the pulmonary artery with a subsequent fatal hemorrhage.

There are two points which seem of some importance in differentiation. The first is the absence of perifocal inflammation, and the second the unusual clarity with which the band area is found in pneumoconiosis. I think if the lungs are examined by coronal section you will find in pneumoconiosis the greatest amount of disease in the band area, relatively little at the base, and very little at the apex.

(Dr. Esmond R. Long, Philadelphia.) I am interested in the amounts of silica you see in the monocytes and in the epithelioid cells that develop from them.

Are they comparable? Have you any evidence as to whether silicate has been formed from the silica in the epithelioid cells? Possibly evidence may be obtained from such a technic as microincineration. The epithelioid cell, the characteristic cell of tuberculosis, represents the response of the body to substances with special physicochemical properties, like the lipoids of tubercle bacilli. The cytoplasm is essentially an emulsion of protein, lipid and water, and here it appears to be possible that we have an emulsion of protein, water and silica.

(Dr. Ellis Kellert, Schenectady, N. Y.) In view of the medico-legal implications of what Dr. Gardner has told us, may I ask if he feels that the presence of silica in the lungs stimulates the progress of tuberculosis, and what effect does it have on the picture of tuberculosis in the lung?

(Dr. Gardner, closing.) Dr. Belt has commented on the difficulty of distinguishing tuberculous lesions from those produced by silicosis. The typical reactions to tubercle bacilli and to silica present no particular problem in diagnosis. Small sections of isolated lesions may be more troublesome. It has been my opinion that probably a great many of the healed tubercles one sees in lungs and peribronchial lymph nodes are influenced by silica particles which tend to gravitate toward these lesions and are retained in their outer walls. This dust modifies the fibrous tissue of the tubercle. We do not generally see healed tubercles in other organs, such as the spleen, with the same kind of hyaline fibrosis arranged in definite laminae that are encountered in lung tissue. Since it is common knowledge that carbon tends to be deposited about immobile structures like tuberculous scars and since it is well known that the amount of silica in the ash of living tissue increases with advancing age, it seems quite probable that silica may accumulate in the periphery of tubercles and produce the hyalinized fibrosis.

As to the question of the necessity of an element of infection in the silicotic lesion, I may say that I am now firmly convinced that silica alone can produce nodular fibrosis. When we first began to discover such reactions in guinea pigs inhaling silica over long periods of time we were amazed at the close resemblance the foci bore to tuberculosis and thought that possibly we were dealing with accidental superimposed tuberculous infection. We therefore skin-tested 200 guinea pigs but never found a positive reaction.

To exclude other infections, we have made Gram stains in an attempt to discover any organisms that might be present in the lesions, and have subinoculated and cultured them, but have never found bacterial infection entering the picture except in cases of obvious pneumonia. One can produce necrosis and fibrosis by injecting sterile suspensions of silica particles into the subcutaneous tissues, and after intravenous injection of pure silica animals develop typical silicotic nodulation in the bone marrow, liver and spleen.

Dr. Reichle brought up the question of cavities in the silicotic lung. In my experience one is apt to see cavities in the so-called conglomerate massive fibrosis of silicosis which are produced as a result of deposition of inhaled silica in portions of the lung previously injured by infection. Perhaps they are unresolved pneumonias similar to those which Dr. Haythorn described in the anthrasilicosis from his locality. Occasionally the cases of this type show central cavity-like areas of degeneration, but the antra do not have definite walls or trabeculae and they do not look like tuberculous cavities. On the other hand, there are cases of perfectly typical tuberculosis associated with silicosis with cavities in which one cannot demonstrate the tubercle bacilli. We have inoculated the cavity contents in a good many cases that showed typical tuberculous histology and I can

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recall about 5 in which the guinea pig failed to develop tuberculosis. We have had the same experience in guinea pigs exposed to silica and infected with tubercle bacilli. The infection would progress and would eventually kill the animals, but sometimes subinoculation of their tissues failed to demonstrate living organisms. I had thought for a time the organisms had lost their acid-fastness in the presence of silica but now I am not sure this is true.

Dr. Reichle spoke about a band across the lungs where silicotic and other lesions tend to localize. I would disagree with him, for I believe that silica or any foreign particles borne in the inhaled air are distributed uniformly throughout the lung. We fail to see evidence of the reaction to silica as nodules both in the bases and sometimes in the apices of the lung because of the emphysema which develops subsequently and displaces the normal relations. The characteristic picture of the silicotic lung shows clear costophrenic regions due to the emphysema which comes on after the nodules have developed in the rest of the lung.

Dr. Long asked about silicate formation inside the monocyte. I do not know whether silica dissolves and recombines with bases in their cytoplasm. Dr. Irwin, who is sitting beside me, will possibly care to discuss this question, as it is in line with some of the work that he has done.

Dr. Kellert asked about the stimulation of tuberculous infection by the presence of silica. We have plenty of experimental evidence and a great deal of clinical evidence to indicate that silica dust does reactivate latent tuberculous foci and cause them to become progressive. Probably 60 to 75 per cent of persons with silicosis ultimately die of tuberculosis. What proportion are infected from exogenous sources after the development of the silicotic nodulation is not known; probably many of them are due to reactivation of preexisting latent foci. Other types of dust do not have the same stimulating effect on tuberculous infection.

**TUBERCULOSIS OF THE PROSTATE.** Robert A. Moore, New York City.

*Abstract.* The prostate in 163 cases of tuberculosis has been studied by the step-section method. A total of 20 instances of tuberculosis of the prostate was found. Correlation with the presence of tuberculosis in other urogenital organs gives no support to the theories of ascending or descending tuberculosis of either the genital or urinary tract. There is strong pathological evidence that tuberculosis of each of these organs is hematogenous except in a rare instance.

**PATHOLOGICAL FEATURES FOLLOWING SHOCK WITH DELAYED DEATH.** Virgil H. Moon and (by invitation) David R. Morgan, Philadelphia, Pa.

*Abstract.* Physiological features compiled from experimental studies on shock may be combined into a useful definition. Shock is a circulatory deficiency, neither cardiac nor vasomotor in origin, characterized by decreased volume of blood and cardiac output and increased concentration of the blood. Regularly associated with it are low blood pressure and low basal metabolism. The coagulability of the blood, the blood chlorides and the renal excretions are diminished. The cardiac rate and the non-protein nitrogen of the blood are increased. Variations in respiratory rate, temperature and some other phenomena are frequent, but not consistent.

This syndrome is accompanied by characteristic visible pathological changes. There is diffuse congestion of the viscera, especially of the lungs, the mucous and serous surfaces, the liver and kidneys. Frequently the serous cavities contain blood tinged fluid. Ecchymoses are often numerous and widespread. Microscopic examination shows marked engorgement of the venules and capillaries and

minute extravasations in each of the tissues mentioned. The spleen is usually contracted and relatively anemic. These changes are regularly present when death occurs early. Edema develops when death from shock is delayed somewhat. This is especially marked in the lungs and mucosae.

We have found these changes following death by shock in a wide variety of clinical conditions, including surgical shock, burns, eclampsia, toxic jaundice, intestinal obstruction, poisoning with barbiturate compounds, and following severe infections.

Several of these conditions were reproduced experimentally by implanting muscular substance into the peritoneal cavity, by cutaneous burns, by intestinal obstruction, by injections of bile and bile salts, by barbital poisoning, and by injections of histamine. Circulatory failure and hemoconcentration preceded death in each instance. Pulmonary edema was a prominent feature in these experiments. The edema fluid had a specific gravity approximating that of the blood serum. When death was delayed several days, secondary pneumonia developed regularly in the edematous lungs. This had the same gross and microscopic features that we have reported (*The Origin of One Type of Secondary Pneumonia*, *Am. J. Path.*, 1933, 9, 899) occurring under similar conditions in man.

### *Discussion*

(Dr. Walter Cannon, Boston.) I have been much interested in the results which Dr. Moon has shown. During the World War we came upon the phenomenon of hemoconcentration, and found in some cases of shock the blood count went as high as 7,000,000 or more per cmm. I was interested also because of the evidence he offered as to a mystery that has confronted persons who have worked on the problem of shock for many years. It has been clear for a long time that the central phenomenon in shock is a diminution of blood volume, usually in severe cases to such a degree that less than the minimal capacity of the circulatory system is present. With this diminished blood volume the blood pressure necessarily falls. The query has always arisen, where is the lost blood? That is sometimes spoken of as "the mystery of the lost blood." The concentration of the corpuscles found in the skin and also in the capillaries of the intestinal villi indicates that there must be a corresponding plasma somewhere. Dr. Moon's evidence as to its extravasation in the places he described this afternoon is a solution, or a partial solution, of this problem.

(Dr. David Perla, New York City.) I have had considerable experience in shock produced by histamine injections in rats over a number of years, and I can fully confirm Dr. Moon's observations, although I have not systematically studied that phase of the problem. I always noted that the animals which died following the injection of histamine, particularly if they died later than a few hours after injection, showed effusions in the pericardial sac and in the peritoneal and pleural cavities. Under these circumstances the blood always had a definite inspissated appearance: it was markedly viscid. I think Dr. Moon's suggestion that there must be a universal change in the permeability of the capillaries and a relative concentration of the red blood cells in the circulating blood is true.

(Dr. Harry Goldblatt, Cleveland.) I should like to ask Dr. Moon just what criteria he has used for the determination of the existence of hemoconcentration and would be pleased if he would define this word as he uses it. The mere presence of an increased number of red blood cells and a corresponding increase in the hemoglobin are not proof of hemoconcentration in the true sense of the word.

Concentration of the blood due to loss of water would be accompanied by changes in the concentration of serum protein and of other substances contained in the plasma. The injection of peptone intraperitoneally produces an effect identical with that which follows the injection of chopped muscle mentioned by Dr. Moon. There is a great increase in the number of the red blood cells and a corresponding increase in the amount of hemoglobin, but there is no other evidence of hemoconcentration. The effect may result from the sudden entrance of red blood cells from a storehouse, such as the spleen, and need not be due to loss of water from the blood.

(Dr. Paul Klemperer, New York City.) I should like to ask Dr. Moon if he examined the liver in his experiments. In his last paper he mentions edema of the liver in only one of his dogs. I am very much interested in this question because in my presentation later to-day I will deal with a related topic. We had only 3 cases of surgical shock in which we examined the liver particularly and did not find edema.

(Dr. Paul R. Cannon, Chicago.) Dr. Holck and I have observed in rats injected with nistol and pernocton that the "delayed death" which occurs at about the 5th day after injection may be explained by certain fatty degenerative changes in the lungs, myocardium, liver and kidneys. Most of the animals developed pulmonary edema and pneumonia, and I was particularly interested in the fatty degeneration of the walls of blood vessels in the lungs. There was obviously a profound toxic injury to the vascular walls, and this injury may be similar to that described by Dr. Moon in explaining "medical shock" as due to capillary atony and increased capillary permeability with hemoconcentration.

(Dr. Walter Cannon, Boston.) So far as the term hemoconcentration is concerned, I am not defending it. I took it over from Dr. Moon temporarily. What we reported in 1918 was a concentration of the corpuscles; we did not go beyond that. We made comparisons of blood taken from peripheral areas in various parts of the body and blood taken simultaneously from the veins of persons in shock. There was a marked discrepancy between the concentration of the corpuscles in the two regions. The corpuscles in the skin areas were very much more concentrated than those taken from the veins. We made no observations on whether there was a loss of water from the blood and therefore a concentration of blood proteins.

(Dr. Moon, closing.) I am deeply gratified by the discussion and the interest shown and am indebted particularly to Dr. Cannon for his interesting remarks. Many of the phenomena we have reported were those observed by him and his colleagues during the World War.

As to the presence of effusions, they are regularly present in acute shock. If the shock is somewhat delayed, we find fewer effusions in the serous cavities and more edema in the lungs and mucosae, but in acute shock, fatal in 24 hours, it is common to find a very marked effusion in the serous cavities. The fluid is blood tinged in the majority of instances.

Regarding the problem of the lost blood, I recall one instance in which shock terminated fatally in 36 hours. The lungs weighed 1200 gm. above normal, due to congestion and edema, and there were over 1500 cc. of bloody fluid in the serous cavities, to say nothing of the edema of the mucosae and other tissues. Those two locations accounted for the loss of almost 3 liters of fluid from the circulation. The lost blood in shock may be accounted for by the effusion, edema and visceral congestion.

Regarding the criteria for hemoconcentration, our interpretation is that which

Dr. Cannon has just made, an increase in the number of corpuscles per cmm. of blood. It may be determined by the blood count or hematocrit.

I am glad Dr. Goldblatt mentioned the effect of peptone, because we did not have time to mention all the agents which will produce shock. A list of the agents causing shock would include peptone and tissue extracts, as well as various poisons. The presence of a higher concentration of the blood in peptone poisoning provides another instance of what we have already stated.

In reply to Dr. Klemperer, the liver in shock is characteristically congested and sometimes edematous. The liver will drip blood freely when sectioned and pathologists have interpreted it, perhaps incorrectly, as passive congestion of the liver. The same statements apply to the kidneys.

I have not looked for fatty degeneration of vessel walls. We were concerned with the edematous, hemorrhagic and congestive features and have not gone into the more minute details of changes in the endothelial cells in shock.

#### THYROTROPIC EFFECT OF CRETIN RATS' PITUITARIES CONSIDERED IN RELATION TO PITUITARY HISTOLOGY. Isolde T. Zeckwer, Philadelphia, Pa.

*Abstract.* The pituitaries of cretin rats were injected into young female guinea pigs on 3 or 4 successive days, and the results compared with the effect produced by similar injections of pituitaries from normal rats of the same age and sex. The histological hyperplasia of the guinea pig thyroids was about the same after pituitary cretin injections as after normal pituitary injections. The absolute weights of thyroids were increased after cretin pituitary injections, but were slightly less than after normal pituitary injections. If calculations are made for the body weights of the rats, the dwarfed cretin has available more hormone for each gram of its body weight than the normal rat. If calculations are also made for the weights of the injected pituitaries, each milligram of the heavier pituitary of the cretin contains about the same amount of hormone, per gram of body weight, as each milligram of the normal.

The cretin pituitary contains large numbers of cells with fine blue granulations and intracellular hyaline material which are histologically very different from "castration cells" which contain coarser, darker granules, with intracellular hyalin of a different character. In pituitaries of rats both thyroidectomized and castrated at the same time, the two types of cells can be differentiated. The cells that react to thyroidectomy may be the producers of the thyrotropic hormone. The intracellular hyaline material, however, seems in excess of any increase in amount of thyrotropic hormone calculated by assay.

#### *Discussion*

(Dr. David Seecof, Montreal.) Perhaps this point was presented, and I may have missed it. I should like to inquire about the time factors in relation to the hyaline droplet cells. How soon after the experiment did they appear, and how long after did they remain?

(Dr. H. E. MacMahon, Boston.) In a recent paper with the title, "A Change in the Basophil Cells of the Pituitary Gland Common to Conditions which Exhibit the Syndrome Attributed to Basophil Adenoma," Dr. Crooke of England demonstrated hyaline changes in the basophil cells of the pituitary gland which he considered to be an expression of altered physiological activity. It was the only conspicuous abnormality common to all twelve examples of basophilism

and he regarded it as the one abnormality of fundamental significance. I should be very much interested in knowing whether Dr. Zeckwer has had an opportunity of comparing the hyaline changes which she has described with those recently reported by Crooke.

(Dr. Zeckwer, closing.) The hyaline material appears in a few weeks time. It is well developed in a month. The last slide of the pituitary was from a 44 day rat, and you see about half of the pituitary is filled with hyaline material. We have studied them up to a year, and up to a year the hyaline material remains. The most pronounced changes are earlier, from a month to 2 to 3 months.

In reply to the second question, I saw Dr. Crooke's sections of pituitary when he came to Philadelphia on a visit. I did not see any of his sections of Cushing's syndrome, but I saw those from cases of Addison's disease in which there were basophilic cells with very small vacuoles in them, not at all like the cells in these cretin pituitaries. The basophilic cells were very different, having a sort of foamy, fatty vacuolated appearance. I have not seen sections of Cushing's syndrome.

#### THE RELATION OF THE HYPOPHYSIS TO THE SPLEEN. David Perla, New York City.

**Abstract.** Removal of the hypophysis in adult rats is followed by progressive atrophy of the spleen. At the end of 2 months the ratio of spleen weight to body weight is one-half the normal. The administration of hypophyseal emulsion repairs to a considerable degree the atrophy of the spleen in such animals.

Hypophysectomy completely inhibits the regeneration of splenic tissue after partial splenectomy. Administration of anterior hypophyseal emulsion restores to normal the regenerative capacity of splenic tissue of the hypophysectomized rat.

The daily administration of anterior hypophyseal emulsion of cattle or of alkaline extracts of fresh or acetone-dried anterior hypophysis during a period of 10 days in normal *Bartonella*-carrier or *Bartonella*-free rats results in hypertrophy of the spleen to twice the normal size. Normal rats receiving emulsion during a period of 1 month become refractory to the spleen-stimulating effect. The spleen shows little increase in size above the normal at the end of this period. Injections of horse serum or of alkaline extracts of acetone-dried kidney, and spleen or liver of cattle do not cause enlargement of the spleen of rats from *Bartonella*-free stock.

The increase in the size of the spleen, following daily administration of emulsion of the anterior hypophysis, is due primarily to a marked hyperplasia of the reticular and endothelial cells of the red pulp. The follicles also increase in size. Clusters of reticular cells containing numerous lymphoid elements appear throughout the splenic pulp. The reticular tissue of the bone marrow is similarly increased. There is a striking increase in the number of hemocytoblasts and megakaryocytes. The Kupffer cells are not affected.

The spleen-stimulating factor is not present in the acid extract of anterior hypophysis that contains thyrotropic and adrenotropic factors. It is present in some degree in an alkaline extract of fresh anterior hypophysis containing growth and gonadotropic factors. It is also present in alkaline extracts of acetone-dried anterior hypophysis relatively free from growth hormone.

The presence of a spleen-stimulating factor in the anterior hypophysis is suggested by these experiments.

*Discussion*

(Dr. Thomas H. Belt, Toronto.) Did you make observations on other organs beside the spleen in these experiments? What calls that to mind is the syndrome known as Simmond's disease in the adult human, where there is a destruction of the anterior lobe of the pituitary and in which other organs are nearly always reduced considerably in size, and conditions in which there is an over-production of the anterior lobe hormones, as in acromegaly, where nearly all the organs are increased in size.

(Dr. Perla, closing.) I do not understand if you meant in the hypophysectomized animals. In them, of course, changes in other organs than the spleen are observed. Many have been reported by numerous investigators, particularly by Smith, who has demonstrated atrophy of all the endocrine glands following removal of the hypophysis, with a corresponding shrinkage in the organs in proportion to body weight. Conversely, the hepatomegaly associated with acromegaly is very variable. In our animals that received alkaline anterior hypophyseal extracts (I did not have time to stress the effect on the other tissues) there did not seem to be produced a corresponding change in the kidney, thyroid or adrenal glands. There was some increase in the gonads of the animals when such alkaline extracts were given.

THE IDENTIFICATION OF TUMOR CELLS IN SEDIMENTS OF SEROUS EFFUSIONS.  
Nathan Chandler Foot, New York City.

*Abstract.* Two methods are commonly employed for identifying tumor cells in serous effusions: (1) The sediment is smeared out and stained in the dry, fixed, or moist state in the usual way, or supravitaly in fresh smears. This does not concern us here. (2) Fluid is sedimented by gravity and the concentrate is then centrifugated and the resulting button fixed, embedded in paraffin and sectioned like tissue. This is the method first published by Mandelbaum in 1900, and discovered and revived by many of us, as Graham has detailed in his article in the *American Journal of Pathology*, 1933, 9, 701.

Zemansky reviewed the results obtained by this method at the Mount Sinai Hospital in New York and formulated criteria for making positive diagnoses of tumor cells, using their experience and drawing on the meager literature. According to him, tumor may be inferred from the presence of: (1) fragments of tissue with a definite arrangement of cells and stroma; (2) multiple clumps of large, deeply staining cells which give the slide a mottled appearance; and (3) finer cellular changes, such as extreme irregularity in size and shape, eccentricity of the nucleus, extremely large nuclei and nucleoli, multinucleation and typical or atypical mitotic figures.

Dissatisfied with the results we were obtaining at the New York Hospital, I undertook to review our series of 55 specimens of ascitic and 85 of pleural effusions. Pericardial fluids had been examined but were uniformly negative. The analysis consisted of tabulating the results of the original examinations as based on clinical history, morphology, and the criteria just stated. This tabulation was followed by a check-up on morphological grounds alone, using the same criteria, but neglecting the clinical history and ignoring the name of the patient, which might be familiar and afford a clue. Special stress was laid on the reliability or unreliability of the morphological criteria. It was found that mitoses occurred in 26 per cent of the slides examined and tumor fragments in 15 per cent. Typical mitoses were, then, found in definitely tumor-negative sections, although

monster or atypical mitoses were never found except in positive cases. Since multinucleation was observed in 65 per cent, irregularity of outline and staining in 43 per cent, and clumping of cells in 54 per cent, these criteria are not of much value.

It was found that desquamated mesothelial cells formed the chief source of error, being easily mistaken for tumor cells. The  $N/n$  ratio, lately stressed by MacCarty in this country and employed somewhat differently by Quensel and Karp abroad in connection with smeared sediments, was worked out in 44 sections checked by biopsy or autopsy. Using Quensel's simple method of dividing the longest nucleolar diameter by the longest nuclear, figures were obtained that completely confirm those he and Karp had found. An  $n/N$  ratio below 0.20 indicates no tumor present; one of 0.25 plus is indicative of a tumor-positive diagnosis; there is a "no-man's land" between 0.20 and 0.25, where the other criteria are also unreliable and the best one can do is to sum up the lot and draw tentative conclusions. Given the other way around,  $N/n$ , by dividing the nuclear diameter by the nucleolar, 0.20 becomes 5:1; 0.25 becomes 4:1, and so on. The ratio of the squares of these figures (roughly approximating their area) is obvious; the cubes, approximating volume, are 125:1; 64:1; and, in the case of our highest figure of 0.40, 15.6:1. Various methods are used for working out the areal or volumetric ratios. As the mere use of the longest diameters gives correct diagnoses in about 70 per cent of the cases, without any other data whatever, it may be inferred that this would suffice. Karp used the formula for calculating the area of an ellipse, taking the longest and shortest diameters of nucleus and nucleolus. His figures do not differ materially from Quensel's.

Summing up, we may say that the following criteria have been found useful: the presence of tumor fragments with stroma, monster mitoses, extreme irregularity in size and shape of the cells in question, the nucleo-nucleolar ratio and a liberal background of clinical data and judgment of morphology obtained through experience.

Normal mitoses, cell clumping, eccentricity of nucleus or multinucleation have been found to be unreliable criteria.

An  $n/N$  ratio of 0.20 or under (Quensel), or 1:125 by the volumetric method (MacCarty), indicates the absence of tumor cells; one of 0.25 or over or 1:64, by the volumetric method, is almost pathognomonic of the presence of tumor.

Lymphoid tumors are diagnosed best by morphological means, as are the myeloid; the  $n/N$  ratio is of chief value in the case of carcinoma.

### *Discussion*

(Dr. Shields Warren, Boston.) I wish to express my thanks to Dr. Foot for this clear exposition, because we are frequently called upon to try to settle this very puzzling problem. I have myself relied somewhat on the concomitance of red blood cells and wonder if he found them of importance in the course of his investigation.

(Dr. David Seecof, Montreal.) In 1924, in a publication of the New York Pathological Society, by relying on the criteria of malignancy such as we all use, I showed that about 70 per cent of the diagnoses were correct. Without using any special criteria, the figure arrived at was practically the same as Dr. Foot just gave.

(Dr. Howard T. Karsner, Cleveland.) I wish to ask Dr. Foot one question, and it must not be interpreted as showing any lack of appreciation of the

thoughtful care and precision used in this work. The question is this: If the findings of the nuclear-nucleolar ratio are in favor of tumor and all the other findings fail to support that conclusion, what diagnosis can be made?

(Dr. Foot, closing.) In reply to Dr. Warren's question as to the red blood cells, we usually add enough glacial acetic acid to our fluid to make it up to 2 to 3 per cent. I have been trying to check up on the point he brings up and found a surprising number of red blood cells in negative fluids, so that I do not think they are of very great importance.

In regard to Dr. Seecof's remarks, Zemansky's percentage was very much better in positive cases of tumor and very much worse in cases that he diagnosed as negative and which were proved to be positive. We have at least improved the negative side of the picture, and I think the whole thing depends on the recognition of changes that take place in the mesothelial cells, which apparently grow rather luxuriously in ascitic and pleural fluid, such fluid acting as a sort of nutrient medium for them, as we often find mitotic figures and other definite qualities of tumor cells.

As to Dr. Karsner's question, that situation sometimes arises, and at the present moment constitutes something of a quandary. I think that if we had a careful negative clinical history and all our morphological data favored a negative report, and if the nuclear-nucleolar ratio favored a positive report, we should certainly scrap the ratio in that case. I am not trying to advocate this ratio as a single method. We must apply all the methods we have. Even then we will find it hard to make the diagnosis in a great many of the cases which lie in the middle ground and contain a great many desquamated cells.

OSSEOUS METASTASES OF CARCINOMA OF THE PROSTATE, WITH SPECIAL REFERENCE TO THE PERINEURAL LYMPHATICS. Shields Warren and (by invitation) Paul N. Harris and Roger C. Graves, Boston, Mass.

*Abstract.* Skeletal metastasis of prostatic carcinoma has been generally attributed to blood borne emboli, but the disproportionately frequent involvement of the pelvis and lumbar vertebrae cannot be satisfactorily explained on this basis. In 7 cases of carcinoma of the prostate and 1 case of carcinoma of the bladder, all the pelvic viscera and soft tissues, with sacrum, bodies of lumbar vertebrae, and adjacent parts of pelvic bones, were removed in a block. After fixation in formalin, horizontal cuts were made at 5 mm. intervals and large sections made. These cases were supplemented by sections of a number of surgical or other autopsy specimens of prostatic carcinoma.

Perineural invasion was not found in the bladder carcinoma, but was present in all the prostatic tumors. In 6 of the 7 prostatic cases perineural invasion was extensive and could be traced up the sacrum and vertebrae. In the other case spread of the tumor seemed to be by lymphatic embolism. Perineural and lymphatic plexus invasion was seen in the periosteum, and direct invasion of the cortical ostia, by tumor extending in from these perineural masses was seen.

Perineural invasion is not peculiar to prostatic carcinoma, but is conspicuous because of the abundance of nerves in and about the prostatic capsule.

The occurrence of pain in prostatic carcinoma and its bone metastases may be due to perineural invasion.

The importance of lymphatic and hematogenous metastasis is admitted, but it is thought that the perineural lymphatics offer the most probable means of invasion of bone and that the disproportionately high incidence of metastasis to pelvis and lumbar vertebrae is probably due to invasion by this channel.



OSTEOID OSTEOMA. Henry L. Jaffe, New York City.

*Abstract.* In the last 3 years I have seen 11 instances of the bone lesion about to be demonstrated. The principal clinical and radiographic features of this lesion can be summarized as follows:

- (a) The patients were all adolescents or young adults.
- (b) The principal complaint was local pain.
- (c) Uniformly, the lesion originated in spongy bone areas.
- (d) As observed radiographically, the pathological areas were roundish and clearly circumscribed.
- (e) The lesions were small and closely similar in size.
- (f) In every case operation was performed on the assumption that the lesion was an inflammatory one.
- (g) Complete eradication resulted in the eventual disappearance of all symptoms, without recurrence of the local condition.

These features reappear from case to case with such regularity that they clearly seem to constitute a clinical syndrome. This fact, in conjunction with the presence of certain special pathological-anatomical manifestations, has led me to regard the lesion as a distinctive one and to designate it as osteoid-osteoma.

On the basis of microscopic examination of the stages of development of the lesion, it appears that the latter arises from osteoblasts. Irregularly between the osteoblasts, intercellular material develops. In this way, patches of osteoid tissue are formed. In the further progress of the lesion, the osteoid tissue becomes calcified and even converted into atypical bone. In the course of the conversion osteoblasts appear. Sometimes the lesions are rather vascular.

In presenting the material I shall begin with the cases in which the lesion is characteristic, clear-cut, and fully developed. It will then be easier to follow the demonstration of the cases that are more obscure, apparently because they are in an earlier stage of their pathological evolution.

As to differential diagnosis, osteoid-osteoma has no features suggesting (a) an inflammatory origin; (b) origin from an embryonic rest; or (c) that it represents an unfamiliar aspect or healing stage of a giant cell tumor, localized osteitis fibrosa, or cyst.

In closing, I wish to suggest that osteoid-osteoma is possibly the benign counterpart of the malignant osteogenic sarcoma. This is indicated by the behavior of a metacarpal bone tumor which I observed some years ago. This tumor was apparently originally an osteoid-osteoma which, because of incomplete removal, continued to proliferate and began to take on the characteristics of an osteogenic sarcoma.

The details of certain of these cases may be found in the *Archives of Surgery*, 1935, 31, 709.

#### Discussion

(Dr. Kornel Terplan, Buffalo.) Are these tumors identical with the so-called osteoid-chondroma of Virchow? I had an opportunity to examine a tumor removed from the upper end of the femur in a young girl. This tumor was about the size of a small peach. The histological structure resembled closely that seen in the slides exhibited by Dr. Jaffe. As far as I know the surgical removal of this tumor resulted in complete cure; there was no recurrence of the osteoid tumor during the 4 years which have elapsed since.

(Dr. Jaffe, closing.) I read the paper of Virchow which was referred to. He

described a large tumor in the humerus, which he designated osteoid chondroma. He stated that while this tumor offered a better prognosis than osteosarcoma it might become malignant and change to osteosarcoma. In any event, I considered this lesion, which had none of the features peculiar to the condition discussed, as osteoid-osteoma.

HEPATIC EDEMA AND "SEROUS HEPATITIS." Paul Klemperer and (by invitation) Harold W. Keschner, New York City.

*Abstract.* In recent writings Roessle and Eppinger attribute great significance to the exudation of serous fluid into the Disse's spaces of the liver and consider edema of the liver as it occurs in infectious or toxic diseases as the chief manifestation of an acellular "serous hepatitis." They believe the liver parenchyma can be severely impaired in such conditions, either because of compression of the liver cells or because of the simultaneous penetration of hepatocellular toxins. Moreover, they regard the extravasation of plasma as a stimulus for connective tissue proliferation and consider it as the initial phase of severe chronic liver alterations, especially atrophic liver cirrhosis. The conclusions of such prominent investigators were deemed worthy of investigation.

Liver sections of 505 consecutive autopsies were studied and, of these, 79 or 15 per cent satisfied the criteria of "serous hepatitis." The greatest incidence, 42 cases, was found in cardiac and nephritic states which were associated with severe hydremia. A high incidence of positive findings in malignant nephrosclerosis, uremia (other than nephritis), diabetic coma, influenza and Graves' disease was noteworthy.

Contrary to Roessle, a surprisingly low incidence occurred in such infectious or toxic conditions as sepsis, typhoid and scarlet fever, acute lupus erythematosus and gastro-intestinal intoxication. Interesting negative findings were observed in cases of peritonitis, subacute bacterial endocarditis, leukemia and malignancy. Thirty liver sections from normal individuals killed by violence did not show intralobular hepatic edema. None of the positive cases examined revealed early phases of fiber formation. Roessle's hypothesis of the formation of collagen fibers within the serous exudate could not be corroborated in these studies. The present studies failed to reveal any evident injury to the capillary walls or striking necrobiosis of the liver cells due to marked edema. The occurrence of intralobular hepatic edema in a considerable number of cases of cardiac failure was striking. However, it did not occur with such frequency that it might be regarded as merely agonal. Moreover, it was observed in cases presenting no evidence of cardiac failure. In these cases it must be accepted as the result of increased capillary permeability. The differentiation from mechanical edema is difficult. The high incidence of positive findings in malignant nephrosclerosis, uremia, diabetic coma, influenza and Graves' disease deserves further investigation. However, the infrequent occurrence of "serous hepatitis" in most of the infectious-toxic states and the lack of striking parenchymal changes in the positive cases did not permit us to ascribe undue significance to "serous hepatitis" as a pathological entity.

GROWTHS OF PATHOGENIC FUNGI ON MEDIUMS MADE OF HAIR AND SKIN POSSIBLY OF VALUE IN EXPLAINING THE SEQUENCE OF EVENTS IN INFLAMMATION. John W. Williams, Cambridge, Mass.

*Abstract.* The growth of a large number (34) of pathogenic fungi and of 2 saprophytes has been studied on synthetic as well as natural tissue mediums, such

as hair, skin and horn, for which they have selectivity. Studies were also carried out on mediums prepared from the hydrolytic products of hair and skin. It was found that on hydrolysates of hair, even with considerable dextrose, growth was almost entirely subsurface, while with skin it was partially so, in contradistinction to the predominantly surface growths on the usual peptone mediums. Studies were carried out using cysteine as the source of nitrogen in place of hair, and it was found that the growth was almost identical with that on hair, indicating that with hair the cysteine system was probably responsible for the type of growth. Other amino acids must also be considered since some 8 I have studied show much subsurface growth. They, however, do not possess the active oxidation-reduction system of cysteine-cystine and would be expected to play a more passive rôle in pathological and physiological processes. Freedom from protein and other control may also be necessary for activity.

It is suggested that an oxidation-reduction mechanism involving the cysteine-cystine system plays an important rôle in the subsurface nature of growth and probably in the invasive power of these fungi and the inflammations they produce.

The factors involved in the cysteine-cystine medium and those reported for inflammatory processes, as well as for the natural growth of these fungi *in vivo*, are quite analogous. In regard to autolysis, acidity, reducing action, oxygen tension, glycolysis, osmotic pressure, penetration, and so on, there is marked similarity in inflammatory processes and in the cysteine-cystine mediums when growth takes place. In the latter, the host reaction which, of course, so markedly influences results is absent.

The author does not propose that the cysteine-cystine system is the only mechanism playing a part in invasiveness, inflammation and chronicity, but that in skin and hair it appears to be an important factor. In other tissues it may play only a secondary rôle, or no part whatsoever.

### Discussion

(Dr. Norman MacL. Harris, Ottawa.) In view of Dr. Williams' statement regarding the working of the oxidation-reduction process, how does he explain the marked invasiveness of another type of organism — the blastomycetes? I do not recall whether or not he included them in the list shown previously on the screen.

(Dr. Williams, closing.) I have not studied the blastomycetes and do not know about them. They of course may remain chronic a varying length of time. Organisms vary with reference to their ability to overcome barriers and to their selectivity. They probably vary with reference to the oxidation-reduction systems which most influence them. I do not know that I made myself clear but this work is only suggestive. You will note that there was slight surface growth with cysteine-cystine.

In regard to microorganisms, particularly bacteria, I have tried some of them, and my luck has not been especially good.

## READ BY TITLE

THE EFFECT OF ANTISYPHILITIC DRUGS ON THE WASSERMANN REACTION.  
David L. Belding, Boston, Mass.

HYPERTENSION APOPLEXY AND ITS CAUSATION. W. H. Chase, Montreal,  
Canada.

VIRULENCE AND ANTIGENIC STRUCTURE OF SMOOTH AND ROUGH CULTURES OF  
*SALMONELLA AERTRYCKE*. G. M. Mackenzie and (by invitation) R. M.  
Pike, Cooperstown, N. Y.

RENAL CHANGES RESULTING FROM ATTEMPTS TO PRODUCE RENAL SHWARTZ-  
MAN REACTION. Alan R. Moritz and Francis Bayless, Cleveland, Ohio.

GLOMERULONEPHRITIS IN AN OPPOSUM, *DIDELPHIS VIRGINIANA*. Herbert L.  
Ratcliffe, Philadelphia, Pa.

THE MAIN AND ANASTOMOTIC VEINS OF THE ADRENAL. David P. Seecof,  
Montreal, Canada.

THE VALUE OF DIFFERENTIAL DIAGNOSIS IN TUMORS OF THE BASAL CELL  
GROUP. Shields Warren and Olive Gates, Boston, Mass.

THE TREATMENT OF ANIMAL TUMORS WITH CYSTEINE HYDROCHLORIDE. C. L.  
Connor and J. L. Carr, San Francisco.



# THE AMERICAN JOURNAL OF PATHOLOGY

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VOLUME XII

NOVEMBER, 1936

NUMBER 6

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## THE EARLY STAGES OF GLOMERULONEPHRITIS\*

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### INTRODUCTION

The structure of the glomerulus in clinical acute diffuse glomerulonephritis has been studied by various investigators and it is generally agreed that the essential lesion is an increase in the number and size of the endothelial cells with resulting capillary obstruction. Polymorphonuclear leukocytes are found in varying numbers among the endothelial cells, but leukocytes alone do not produce complete capillary obstruction except in occasional capillary loops.

Investigators interested in the pathogenesis of human glomerulonephritis have studied clinical examples of the disease in which death occurred shortly after the onset of symptoms. In general they have found appearances similar to, but less prominent than, those occurring in well developed clinical cases.

Gräff, 1916, using Schultze's oxidase reaction, demonstrated a great increase of polymorphonuclear leukocytes in acute glomerulonephritis. He thought that the number of leukocytes in the glomeruli afforded a distinction between simple inflammatory reactions and acute glomerulonephritis.

Gross, 1919, studied the kidneys of a person who died of pulmonary edema a few hours after the onset of symptoms of nephritis. In the glomerular capillaries he noted leukocytes and many large endothelial nuclei embedded in a cytoplasmic network.

Volhard, 1922; 1931, proposed the theory that the primary change in acute glomerulonephritis is a spasm of the afferent glomerular arterioles, the resulting anemia injuring the capillaries and bringing

\* Received for publication March 28, 1936.

about a secondary inflammatory reaction. He based his hypothesis on clinical and physiological considerations and on analogy with eclampsia.

Kuczynski and Dosquet, 1926, supported Volhard's theory with anatomical evidence. In a case which they considered early acute glomerulonephritis they noted edema of the wall of the afferent glomerular arteriole near its entrance into the glomerulus. Leukocytes were also found in the wall of the arteriole. They believed that this structural alteration indicated a primary spastic constriction of the afferent arteriole. The leukocytes in the glomerular capillaries were supposed to have entered through the efferent arteriole. An increase of endothelial nuclei was described.

Volhard's theory has received very little support from anatomical studies. It is, of course, difficult to refute since any spasm of the arteriole present in life would probably relax after death, and one would not expect arteriolar spasm to produce structural changes in the wall of the vessel. In some cases of glomerulonephritis the arterioles show an occasional area of hyaline degeneration in the media, but it is certain that the vast majority show no anatomical changes. They are frequently dilated rather than contracted at the point of entrance into the glomerulus.

Endothelial proliferation does not seem to be related to hypertension. It will be shown later in this paper that severe infections without hypertension show much more endothelial proliferation in the glomeruli than is found in primary hypertension. The high frequency of endothelial proliferation in association with infectious and toxic processes indicates that it is a response to soluble toxic substances rather than to injury from anemia.

Fahr, 1926, restated his belief that glomerulonephritis is caused by soluble toxins that produce a primary endocapillaritis. In a person who died of pneumonia 3 days after the onset of symptoms of nephritis he found a definite endothelial proliferation and many leukocytes in the glomerular capillaries. A careful study of the afferent arterioles showed no lesions of any kind.

Hückel, 1929, studied the kidneys from a case of glomerulonephritis of 30 hours duration. He found the glomerular capillaries filled with polymorphonuclear leukocytes and endothelial cells in a cytoplasmic substance. He agreed with Fahr's conception of primary endocapillaritis.

The prevailing opinion in the literature seems to be in accord with Fahr's conception that the primary change in glomerulonephritis is proliferation of the endothelium of the glomerular capillaries and that the earliest clinical examples of acute glomerulonephritis show this lesion. The simplest interpretation of the endothelial increase is an inflammatory reaction to soluble toxic substances.

We may now raise the question as to whether or not acute glomerulonephritis is a specific disease entity, *i.e.* a disease caused by a specific organism or virus and having characteristic anatomical lesions, such as typhoid fever and tuberculosis. Inasmuch as organisms are not found in the glomeruli the etiological agent can only be inferred from the associated infection in the patient. Nearly all observers are agreed that the associated infectious agent is usually a streptococcus, but both hemolytic and non-hemolytic strains are concerned. There is also strong evidence that pneumococci may occasionally cause glomerulonephritis, and in rare instances other organisms may be concerned. Staphylococci apparently do not produce diffuse endothelial proliferation in the glomeruli to any notable extent although they often produce glomerular abscesses. It may be concluded, therefore, that glomerulonephritis is not caused by a specific etiological agent.

As regards the anatomical lesion, the essential feature is proliferation of the glomerular capillary endothelium. Polymorphonuclear leukocytes are usually present in varying numbers but are not an essential feature. Leukocytes alone rarely, if ever, produce widespread capillary obstruction. Epithelial crescents predominate in certain fulminant cases and may be largely responsible for obstruction of the glomeruli.

In 1926 Clawson, Bell and Hartzell published the observation that a moderate degree of diffuse glomerulitis is frequently found in persons dead of subacute bacterial endocarditis. Since that time I have noted a similar glomerulitis in various acute infectious processes, notably puerperal septicemia. These subclinical forms of diffuse glomerulonephritis were illustrated in my "Text-Book of Pathology" in 1934 (Figs. 217 and 218).

In this paper the study of the *finer glomerular structure* has been extended to include a large number of infectious and non-infectious diseases. It will appear from this investigation that a diffuse proliferation of the glomerular endothelium occurs in many acute and



chronic infectious processes and in some diseases in which no infectious element was found. There are innumerable transitions between this subclinical glomerulitis and clinical acute glomerulonephritis. The clinical disease shows an increased endothelial proliferation and more pronounced capillary obstruction, but fundamentally it is the same type of reaction that occurs in the subclinical forms.

If this interpretation be correct, *i.e.* that subclinical and clinical acute glomerulonephritis differ only in intensity, it follows that acute glomerulonephritis is not a sharply circumscribed entity and a much broader approach to its etiology and pathogenesis is available.

#### MATERIAL AND METHODS

In this investigation the kidneys from 865 cases have been examined microscopically (see Table II). A wide range of diseases is included and all age groups are represented. Paraffin sections were stained by the Mallory-Heidenhain technique (azocarmine). This stain was first applied to the glomeruli by McGregor. Since the capillary basement membrane is stained sharply, one may easily distinguish cells within the capillaries (endothelial cells and leukocytes) from the glomerular epithelial cells outside them. The stain works to best advantage on tissues fixed in Helly's or Zenker's fluid, but with slight modifications it is satisfactory after formalin fixation unless the tissues have been in formalin for over a year. Tissues kept in formalin several years may often be stained satisfactorily if the deparaffined sections are treated with Zenker's fluid for 24 hours.

In estimating the degree of endothelial proliferation the number of endothelial and epithelial nuclei in several glomerular loops are counted. When the epithelial nuclei definitely outnumber the endothelial the degree of endothelial proliferation is graded "0," and when the two types of nuclei are of approximately equal number it is graded "+." A definite preponderance of endothelial nuclei is graded "1" and a marked preponderance "2." In Grades 1 and 2 the endothelial nuclei are large and show abundant cytoplasm about them. Grade 3 endothelial proliferation corresponds with the structure seen in typical clinical acute glomerulonephritis. Intracapillary fibers are easily seen in Grade 3, and occasionally in very small amount in Grade 2, but are entirely absent in lower grades of proliferation.

In counting the nuclei within the capillaries polymorphonuclear leukocytes were not included, but mononuclear leukocytes were counted as endothelial cells unless they lay entirely free in the capillary lumen. It is very difficult to distinguish a mononuclear leukocyte from a large endothelial cell when the former is flattened against the capillary wall.

This method of enumerating the endothelial nuclei does not, of course, give the total number of cells in a given volume of glomerular tissue and it assumes that the number of epithelial cells is fairly constant.

It is true as Van Waveren points out that fewer endothelial nuclei are visible in a section when the capillaries are distended than when they are empty. In a normal glomerulus, where the endothelial nuclei are smaller than the epithelial, this may lead to underestimation of the relative number of endothelial cells; but in all forms of glomerulitis the endothelial nuclei are as large as the epithelial, and therefore distention or collapse of the capillaries does not alter the ratio of endothelial to epithelial nuclei.

Grades 1 and 2 of endothelial proliferation are immediately recognizable with the high dry lens. Figures 3, 4, 5 and 6 illustrate what is meant by endothelial proliferation — 0, +, 1, and 2 respectively.

#### THE STRUCTURE OF THE NORMAL HUMAN GLOMERULUS

In a normal glomerulus the lobulation is usually indistinct since the interlobular fissures are difficult to see. However, in chronic glomerulonephritis the lobules are often shrunken so that the interlobular fissures become conspicuous (Figs. 1 and 2). In midsagittal sections through the vascular pole of such slightly shrunken glomeruli one sees from three to five fissures that penetrate nearly to the vascular pole. Some of the primary lobules thus formed are subdivided peripherally by fissures which extend from one-third to one-half the distance to the vascular pole and form secondary lobules. Capillary loops bulge from the surfaces of the secondary lobules to form small tertiary lobulations. The tertiary lobules are indistinct in the shrunken glomerulus (Fig. 1) but are easily seen in a normal glomerulus. In tangential sections through a glomerulus one sees small isolated, secondary or tertiary lobules, each composed of one or more capillary loops surrounded by epithelium.

Judged from the appearances seen in sections through different

planes one may conclude that there are from four to six primary lobules of irregular conical shape with their apices near the vascular pole and their wide bases at the surface of the glomerulus. Each primary lobule branches distally into secondary and tertiary lobules.

It is clear that the elaborate separation of the glomerulus into lobules serves the purpose of bringing nearly all the capillaries into contact with the surface. There are few capillaries that are not in contact with the surface epithelium at some part of their circumference, and those in the small peripheral tertiary lobules are usually largely or completely surrounded by epithelium. The glomerular epithelium covers the surface of the glomerulus and lines all the shallow intralobular as well as the deep interlobular clefts. There are obviously no capillary anastomoses across any of the fissures.

The glomeruli in newborn infants are smaller and of less complex structure than those of adults. The primary lobules are smaller and secondary lobulation is much less conspicuous.

The finer structure of the glomerulus is best seen in the small tertiary lobules at the surface. These consist of a few capillary loops closely invested by glomerular epithelium (Fig. 3). The epithelium covers the outer surfaces of the capillaries and fills the interstices between them. The nuclei between capillaries probably all belong to epithelial cells.

### *The Glomerular Epithelium*

The glomerular epithelium is the visceral epithelial layer of the capsular space and is continuous around the vascular pole with the capsular epithelium (the parietal epithelial layer of the capsular space). Both the parietal and the visceral epithelial layers of the capsular space have the same embryonic origin as the cells of the convoluted tubules, since the capsular space is formed by invagination of the glomerular tuft into the expanded end of the primitive tubule. This relation is seen in tubular disease of the kidneys in which one may see hyaline granular degeneration, fatty degeneration and necrosis of the glomerular and capsular epithelial cells when these changes are present in the tubular epithelium.

As pointed out above, the epithelial layer lines all the interlobular and intralobular clefts and penetrates into the lobules between the capillaries. It therefore forms a support for the capillaries as well as an external covering. The epithelial cells are much more conspicu-

ous in some kidneys than in others. In infants the surface layer is columnar or cubical in shape and very conspicuous. In adults the cytoplasm of the epithelial cells is sometimes so abundant that the cells seem to compress the capillaries. More often, however, their nuclei are found in the interstices between the capillaries and sparsely distributed over the surface. Wide areas of the capillary surfaces may appear to be denuded of epithelium, but a careful study will always reveal a layer of epithelial cytoplasm separating the capillary basement membrane from the capsular space (Fig. 3). The surface epithelial layer undergoes postmortem autolysis rapidly, and the number and size of the cells are underestimated in poorly preserved tissue.

The parietal epithelium of the capsular space (the capsular epithelium) plays an important rôle in glomerulonephritis, since it is the source of the epithelial crescents; but the glomerular layer shows chiefly degenerative changes and never proliferates sufficiently to compress the glomerulus.

### *The Glomerular Endothelium*

McGregor, 1929, has described and illustrated the finer histology of the normal glomerulus. In preparations stained by the Mallory-Heidenhain method the endothelial nuclei are easily distinguished from the epithelial by their position on the inner surface of the capillary basement membrane. In a large majority of normal kidneys the structure of the glomerulus corresponds to that shown in Figure 3, which I have called Grade 0. The epithelial nuclei greatly outnumber the endothelial. Practically no cytoplasm is seen about the endothelial nuclei or elsewhere on the inner surface of the basement membrane.

In Table I the degree of endothelial proliferation in 107 normal kidneys is recorded, the cases being arranged by decades. The kidneys classified as normal were from individuals who died of trauma or carbon monoxide poisoning not more than 6 hours after the injury was sustained. The majority lived less than 1 hour after the accident. A further requirement was that there should be no gross or microscopic evidence of any disease other than the fatal trauma or poisoning.

It will be noted from the table that 90 of the 107 cases showed Grade 0 endothelial proliferation, which indicates that the epithelial

nuclei were more numerous than the endothelial. A definite preponderance of epithelial nuclei is illustrated in Figure 3. In 16 of the 107 cases epithelial and endothelial nuclei were present in approximately equal numbers. This type of structure is graded + and is illustrated in Figure 4. Inasmuch as about one-seventh of the normal kidneys showed Grade + structure, this must be accepted as a variation within normal limits.

TABLE I

*The Endothelium of the Glomerular Capillaries in Apparently Normal Kidneys \**

Decade	No. of cases	Endothelial proliferation		
		o	+	1
1	9	9	0	0
2	8	6	2	0
3	17	13	4	0
4	25	21	4	0
5	17	15	1	1
6	11	9	2	0
7	12	12	0	0
8	6	4	2	0
9	2	1	1	0
Total	107	90	16	1

\* In the o column the kidneys are listed in which the epithelial nuclei definitely outnumber the endothelial (Fig. 3). Grade + indicates that the epithelial and endothelial nuclei are approximately equal in number (Fig. 4). Grade 1 indicates a definite preponderance of endothelial nuclei (Fig. 5).

In only one instance did an apparently normal kidney show a definite preponderance of endothelial nuclei. This structure is called Grade 1 endothelial proliferation and is illustrated in Figure 5. Although no disease was found at postmortem to account for this endothelial increase, it is probably beyond the limits of the normal variation in structure.

Nussbaum, 1886, demonstrated cell boundaries in the endothelium of the frog's glomeruli. Bensley and Bensley, 1930, were able to see some cell boundaries in human glomeruli by staining with silver; and Zimmermann, 1933, observed cell boundaries in the glomeruli of cats. However, a majority of investigators have failed to demonstrate cell boundaries in the endothelium of the glomerular capillaries although they found them readily in the afferent arteriole.

There is disagreement in the literature as to the number of endo-

thelial nuclei normally present. Langhans, 1885, found only a few endothelial nuclei. Von Möllendorff, 1927, observed in the human glomerulus only a few endothelial nuclei. A little cytoplasm was found about the nuclei but no cell boundaries were demonstrated. Bargmann, 1929, 1931, and McGregor, 1929, agreed with Von Möllendorff that the endothelial cells are few in number, sparsely distributed and greatly outnumbered by the glomerular epithelial cells.

Borst, 1931, found that endothelial nuclei are more numerous than epithelial except in infants and young children where a reverse relation obtains. Borst used a technique which involves boiling of fresh tissue and results in great damage to the cytoplasm of the epithelial cells.

Van Waveren, 1935, used a technique similar to Borst's except for a short preliminary fixation in formalin before boiling. This method gives good pictures of the basement membrane but destroys the cytoplasm of epithelial cells. Van Waveren estimated the number of endothelial and epithelial nuclei in corresponding volumes of glomerular tissue and came to the conclusion that endothelial nuclei always outnumber epithelial. He presents the interesting hypothesis that even in glomerulonephritis the endothelial cells do not increase in number but only in size. It seems, however, that the boiling method damages the epithelial cells so severely that one may be led to underestimate their number. It is also to be noted that the post-mortems from which this author apparently obtained his material were performed 36 to 42 hours after death. Ordinarily the surface layer of glomerular epithelial cells has undergone extensive autolysis by this time, and one would not expect to find all of these cells still present.

Van Waveren maintains that the appearances which I have described as glomerulitis do not represent an actual increase of endothelial cells but merely an apparent increase due to contraction of empty capillaries. Empty collapsed capillaries, however, usually show a wavy basement membrane and are easily distinguished from glomerulitis.

Wilbur, 1931, studied the kidneys of 25 apparently healthy subjects dead from accidental causes. He found that endothelial nuclei were from four to six times as numerous as epithelial.

My own observations have been recorded above. The epithelial cells were found to outnumber the endothelial in 90 of 107 normals

and usually the former were much more numerous than the latter. In 16 instances the endothelial and epithelial cells were approximately equal in number. My conclusion is that a definite preponderance of endothelial cells with an increase of their cytoplasm, as shown in Figure 5, represents a glomerulitis. It will be shown presently that the endothelial cells often show a great increase in number and size in infectious and toxic processes, and if one examines the kidneys from a large series of consecutive postmortems he will find that a fairly high percentage of them show more endothelial than epithelial cells in the glomeruli. It appears from Table II that 41.7 per cent of the 865 cases studied showed a definite excess of epithelial over endothelial cells, while in 30.4 per cent the reverse relation obtained. In 27.8 per cent the endothelial and epithelial cells were approximately equal in number. The material studied, however, is not a uniform sample of postmortem material since there is an undue proportion of infectious processes. In a corresponding number of consecutive postmortems the percentage with Grade 0 endothelium would doubtless be greater.

TABLE II

*Age Distribution of the Endothelial Patterns in All the Cases Studied, Including the Normals\**

Decade	Endothelium										Total
	o		+		1		2		3		
	No. cases	Per cent	No. cases	Per cent	No. cases	Per cent	No. cases	Per cent	No. cases	Per cent	
0-10 yrs.	42	79.3	6	11.3	4	7.5	1	2.0	0	0	53
10-20 "	18	30.0	19	31.6	18	30.0	4	6.7	1	1.7	60
20-30 "	34	26.6	34	26.6	46	36.0	12	9.4	2	1.5	128
30-40 "	50	34.0	53	36.0	36	24.5	7	4.8	1	0.7	147
40-50 "	74	42.8	48	27.7	43	24.8	8	4.6	0	0	173
50-60 "	56	47.5	23	19.5	35	29.6	4	3.4	0	0	118
60-70 "	53	46.9	33	29.2	22	19.5	5	4.4	0	0	113
70-80 "	23	41.1	23	41.1	10	17.8	0	0	0	0	56
80-90 "	10	..	2	..	4	..	0	..	0	..	16
90-100 "	1	..	0	..	0	..	0	..	0	..	1
Total	361	41.7	241	27.8	218	25.2	41	4.7	4	0.5	865

\* Reading horizontally one sees the number of cases of each endothelial pattern and the percentage of each pattern in the decade. Reading vertically one may compare the frequency of any endothelial pattern in the various decades. Grade 0 indicates that epithelial outnumber endothelial cells; + indicates that epithelial and endothelial cells are approximately equal in number; 1 indicates a definite preponderance of endothelial cells (Fig. 3); 2 indicates a rather marked glomerulitis but somewhat below the clinical stage; 3 indicates a clinical glomerulonephritis.

*The Influence of Age on the Endothelial Pattern*

In Table II the distribution of all the cases studied, including the normals, is shown according to the decades and the endothelial pattern. The accuracy of the percentages may be judged by the numbers on which they are based. In the first decade the percentage with Grade 0 endothelium is very high. In infants and young children this endothelial pattern prevails even in association with infectious diseases. The low percentage with Grade 0 in the 2nd, 3rd and 4th decades is no doubt due to the inclusion of a large number of cases of puerperal sepsis and bacterial endocarditis. It is improbable that age influences the endothelial pattern after the first decade.

TABLE III

*Distribution of the Endothelial Patterns in the Normals and the Various Diseases that were Studied\**

	Num- ber of cases	Endothelium				
		Per cent 0	Per cent +	Per cent 1	Per cent 2	Per cent 3
1. Normals .....	107	84.1	15.0	0.9	0	0
2. Miscellaneous non-infections .....	94	55.0	30.8	14.0	0	0
3. Primary hypertension .....	100	46.0	41.0	11.0	2.0	0
4. Lobar pneumonia .....	112	51.8	29.5	13.4	5.4	0
5. Acute rheumatic endocarditis .....	61	44.0	15.0	38.0	3.0	0
6. Acute bacterial endocarditis .....	37	30.0	27.0	43.0	0	0
7. Subacute bacterial endocarditis ....	85	3.5	17.6	60.0	16.5	2.4
8. Puerperal sepsis .....	84	6.0	41.7	38.0	12.0	2.4
9. Pulmonary tuberculosis .....	73	15.1	45.2	37.0	2.7	0
10. Miscellaneous infections .....	112	36.6	25.9	29.5	8.0	0

\* Explanation as in Table II.

*Effect of Disease on Endothelial Pattern*

*Miscellaneous Non-infectious Diseases (Table III, No. 2):* This group includes a large variety of diseases in which infection plays no rôle except as a terminal complication. The diseases included are as follows: old valve defect, 15; malignant tumors (carcinoma of stomach, pancreas, and so on), 19; pernicious anemia, 7; atrophy of liver, 8; diabetic coma, 7; alcoholism, 5; coronary disease, 4; burns, 3; arsenic poisoning, 2; and 1 each of 24 other diseases. The frequency of the various endothelial patterns is shown in Table III, No. 2. A Grade 1 glomerulitis was present in 13 of the 94 cases (14 per cent).



It was present in the following diseases: pernicious anemia, 4 cases (out of 7 examined); old healed valvular heart disease, 5 cases; 1 case each of subacute atrophy of the liver, subacute combined degeneration of the spinal cord, carcinoma of the ampulla of Vater, and right heart failure. Terminal infections may have played a rôle in causing the glomerulitis but this could not be established conclusively.

*Primary Hypertension (Table III, No. 3):* In this group there were 11 cases of Grade 1, and 2 of Grade 2 glomerulitis. In the 2 cases with Grade 2 glomerulitis death was due to renal insufficiency. The causes of death in the 11 cases with Grade 1 glomerulitis were as follows: myocardial exhaustion with congestive heart failure, 4; renal insufficiency, 2; coronary disease, 2; and 1 case each of cerebral hemorrhage, pyloric obstruction and rupture of the aorta. It is not uncommon to find a definite endothelial increase in hypertension with renal insufficiency — glomerulitis often seems to be an essential part of the renal lesion. Not infrequently patients with primary hypertension die of some complicating infection such as septicemia or pneumonia, and occasionally in such cases clinical acute glomerulitis is found at postmortem; but hypertensives with an obvious terminal infection were not included in this group. The glomerulitis in the 9 cases without renal insufficiency cannot be satisfactorily explained as a result of infection.

We shall now consider the glomerular structure in definite infectious diseases.

*Lobar Pneumonia (Table III, No. 4):* One-hundred-twelve cases of this disease were studied. It is surprising to find that the frequency of glomerulitis is not significantly greater than in the non-infectious diseases. It is true that there are 6 cases of Grade 2 glomerulitis, but over 50 per cent of the kidneys show the Grade 0 endothelial pattern. No correlation could be found between the degree of endothelial proliferation and the duration of the illness or the age of the patient. Apparently pneumococci do not stimulate the glomerular endothelium to the degree that streptococci do.

It is recognized in the literature that clinical acute glomerulonephritis may follow lobar pneumonia, but postpneumonic nephritis is generally believed to be quite rare. Abrahams, 1920, found that acute nephritis developed in only 2 of 558 cases of typical lobar pneumonia. Eliassow, 1920, described a convincing case of acute

glomerulonephritis in a male 38 years of age, who developed symptoms (albuminuria, edema and moderate hypertension) on the 11th day after the onset of pneumonia. The disease ended in recovery about 6 months later. Seegal, 1935, in a study of 1004 cases of lobar pneumonia found that 7 developed acute glomerulonephritis.

McIntosh and Reimann, 1926, studied renal function during pneumonia. They noted that some previous investigators had found a slight decrease and others a slight increase of kidney function. They studied the elimination of phenolsulphonephthalein after intravenous injection, and also determined the index of urea concentration. The kidneys frequently showed an increased functional ability which began before the crisis and persisted for several days after it. No examples of decreased functional power were mentioned.

Neale, 1928, examined the urine of 287 adult patients with lobar pneumonia. In 3.4 per cent the albumin was + + +, in 48 per cent +, and in 47.5 per cent it was absent. No examples of acute glomerulonephritis were found in the 42 postmortem examinations that were made. In 102 cases of pneumococcal infection other than lobar pneumonia, normal urine was found in 46 cases, albumin alone in 45, and albumin casts and blood in 11. In 21 cases of acute pneumococcal infection in children under 7 years of age the urine was normal in 5, contained some albumin in 12, and contained albumin, casts and blood in 4.

Lyttle and Rosenberg, 1929, state that pneumonia in children is frequently followed by nephritis.

Blackman, Brown and Rake, 1931, injected rabbits intravenously with pneumococcal autolysate and intradermally with pneumococci. Eighteen of the rabbits developed generalized edema with ascites. The lesions in the kidneys were interpreted as comparable to human acute and subacute nephritis. These authors also found mild acute and subacute nephritis in 40 to 50 per cent of persons dead of pneumococcal infections. However, there was no anatomical evidence submitted which indicates that any of these renal lesions were true glomerulonephritis. The lesions described were chiefly tubular injuries, thrombosis of glomerular capillaries and occasional epithelial crescents.

Blackman and Rake, 1932, found acute nephritis of considerable intensity in 9.5 per cent of a group of young infants with pneumococcal infections (empyema, organizing pneumonia, otitis media,

meningitis). The diagnoses were made by postmortem examination; no case was recognized as nephritis clinically. They found no cases of nephritis in older children or adults following pneumococcal infections.

Blackman and his associates use the term "nephritis" in a very broad sense to include lesions that are chiefly tubular, and do not restrict it to glomerulonephritis.

The fact that persons suffering with lipoid nephrosis frequently develop pneumococcal peritonitis has led to the belief that pneumococci are responsible for this type of renal disease.

*Acute Rheumatic Endocarditis (Table III, No. 5):* This group includes two clinical types: (a) those dying during the first attack from septicemia or a complicating infection such as pericarditis; and (b) those dying from a recurrent acute attack in which valvular insufficiency was a contributory cause of death. Glomerulitis was somewhat more frequent in the first type. It may be seen in Table III that glomerulitis was present in 41 per cent. Although the group is small this percentage seems significantly higher than in the preceding group. In rheumatic endocarditis there are comparatively few bacteria in the circulating blood and one would not expect to find as much glomerular irritation as in bacterial endocarditis.

*Acute Bacterial Endocarditis (Table III, No. 6):* This group includes primary bacterial endocarditis of less than 6 weeks duration and bacterial endocarditis secondary to some major infection. The duration of the illness is much shorter than in the subacute form. The number of cases is too small to have much significance, but there is a suggestion that infections of this type cause proliferation of the glomerular endothelium.

*Subacute Bacterial Endocarditis (Table III, No. 7):* In this disease, which is nearly always caused by streptococci, there is usually a prolonged bacteriemia and the glomeruli are exposed to large quantities of bacterial poisons for many months. As might be anticipated, the effects on the glomerular endothelium are very striking. Only 3.5 per cent of the glomeruli show the Grade 0 endothelial pattern, as compared with 84.1 per cent in the normals, and 79 per cent of the cases show glomerulitis. The higher degrees of glomerulitis are numerous, and in 2 instances the typical structure of clinical acute glomerulonephritis was present although it was not recognized

as such clinically. Seven cases were omitted from the table because the glomeruli were so extensively involved with embolic lesions that the endothelial pattern could not be determined. There is no correlation between the number and size of the embolic lesions and the intensity of the endothelial proliferation. Many cases of severe diffuse glomerulitis showed no embolic lesions. The glomerulitis in the cases of acute endocarditis mentioned above is much less intense than in the subacute group, but no definite relation could be established in the subacute group between the duration of the disease and the intensity of the glomerulitis. The glomerulitis may be more pronounced in a case of 2 months duration than in one that lasted over 1 year.

In bacterial endocarditis every transition may be found between kidneys that show the normal Grade 0 endothelial pattern and those that show the structure of typical clinical acute glomerulonephritis. In a large series of these cases one may trace the pathogenesis of the glomerular lesions, and the various stages are illustrated in Figures 4, 5, 6 and 7. The more or less constant presence of streptococci in the blood in this disease over a period of several months would lead us to expect a much higher incidence of clinical acute glomerulonephritis; yet 60 per cent of the cases show only Grade 1 glomerulitis. Evidently the development of the clinical lesion depends on some factor other than the presence of streptococci in the blood stream. Baehr and Lande, 1920, found that 9 of 77 cases of subacute streptococcic endocarditis showed diffuse glomerular damage.

*Puerperal Sepsis (Table III, No. 8):* In this disease, as in subacute bacterial endocarditis, there is a high incidence of glomerulitis, 52.4 per cent. Most of these are Grade 1 glomerulitis; but 12 per cent show the Grade 2 pattern, and there are 2 cases of clinical acute glomerulonephritis. This disease is usually due to streptococci, although other organisms, *e.g.* staphylococci, are occasionally responsible. Peritonitis and bacteremia are the usual fatal complications. As in the case of subacute bacterial endocarditis, there are numerous transitions between mild and severe glomerulitis, and the distinction between subclinical and clinical glomerulonephritis is somewhat arbitrary.

*Pulmonary Tuberculosis (Table III, No. 9):* In this group only those cases are included in which the patient died of chronic pulmonary tuberculosis. In all instances the lungs were extensively

destroyed by cavities and tuberculous tissue. It is probable that the high frequency of glomerulitis, 39.7 per cent, is due to the pyogenic infection in the cavities rather than to any toxic products of the tubercle bacillus. Some of these kidneys contain deposits of amyloid. In a previous publication, 1933, I have called attention to the increase of endothelial nuclei that precedes the deposition of amyloid. It appears from the present study that this endothelial increase is the result of the underlying infection and is independent of the formation of amyloid. Occasionally a clinical acute glomerulonephritis follows pulmonary tuberculosis.

*Miscellaneous Infections (Table III, No. 10 and Table IV):* This group includes 112 cases of various infectious processes encountered

TABLE IV

*Miscellaneous Infections Arranged According to the Disease and the Degree of Endothelial Proliferation\**

Infection	Endothelium				Total cases
	0	+	1	2	
Typhoid fever .....	3	2	0	0	5
Septicemia .....	5	4	4	1	14
Peritonitis, appendicitis .....	8	6	7	2	23
Pyelonephritis .....	0	0	2	0	2
Acute and chronic suppuration .....	3	12	9	1	25
Diphtheria .....	6	1	0	1	8
Scarlet fever .....	6	0	0	0	6
Bronchopneumonia .....	2	1	1	1	5
Endarteritis .....	0	0	1	0	1
Septic sore throat .....	1	1	2	0	4
Pericarditis or pleuritis .....	1	2	2	0	5
Meningitis .....	6	0	3	0	9
Acute hepatitis .....	0	0	0	1	1
Lupus erythematosus .....	0	0	1	1	2
Purpura hemorrhagica .....	0	0	0	1	1
Enterocolitis .....	0	0	1	0	1
Total	41	29	33	9	112

\* Explanation as in Table II.

in a series of consecutive postmortems. No instance of clinical acute glomerulonephritis following an infection was encountered in this series of postmortems, but clinical glomerulonephritis is commonly related to such infections. In a group of 57 cases of clinical acute glomerulonephritis collected from a series of 23,000 postmortems there were 25 cases that followed miscellaneous infections of the type

listed in Table IV: 37.5 per cent of this group show glomerulitis, Grades 1 and 2.

In Table IV the group is arranged according to the disease and the endothelial pattern. The surprisingly high incidence of the Grade 0 endothelial pattern is due to the inclusion of 27 cases in children under 10 years of age, 22 of which showed the normal Grade 0 structure. If these 27 cases are excluded the percentage of Grade 0 type drops from 36.6 to 22 per cent, and is then more comparable to the other groups shown in Table III which are chiefly adults. It is to be noted that diphtheria and scarlet fever do not have much effect on the endothelium. Fahr, 1916, found only 1 case of acute glomerulonephritis from postmortem examination of 110 cases of diphtheria. It is known that glomerulonephritis is rarely found in persons who die during an attack of scarlet fever.

*The Relation of Endothelial Proliferation to Albuminuria*

In the 246 cases included in Table V the urine was examined at some time during the fatal illness and usually only a few days before

TABLE V

*The Relation of Endothelial Proliferation to Albuminuria*

Endothelium	Albuminuria				Total
	o or trace		+ to +++++		
	No. of cases	Per cent	No. of cases	Per cent	
o	47	74.6	16	25.4	63
+	41	67.2	20	32.8	61
1	74	71.8	29	28.2	103
2	10	52.6	9	47.4	19
Total	172		74		246

death. The cases in which the urine contained no albumin or only a trace are listed together. If this group be subdivided, there are 100 with no albumin and 72 with a trace. The cases in which albuminuria was graded + to +++++ are listed together, since these are only rough estimations of the amount of albumin. It is obvious that there is no relation between the presence or the amount of albumin and the degree of endothelial proliferation. A few cases with Grade 0 endothelium showed heavy albuminuria, and some cases with Grade 2 proliferation showed no albumin. It may be argued that this

is evidence that the endothelial proliferation is not an inflammatory reaction, but it is well established that albuminuria depends on some injury of the capillary endothelium which makes it permeable to protein. There is more albumin in the urine in lipoid nephrosis, which often shows little or no endothelial proliferation, than in glomerulonephritis with a pronounced endothelial increase.

Cloudy swelling of the kidneys likewise shows no direct relation to increase of endothelial cells, since it may be as pronounced in those with the Grade 0 endothelial pattern as in those with Grade 2. However, those with the Grade 2 pattern always showed cloudy swelling.

### *The Rôle of the Polymorphonuclear Leukocyte*

Gräff, 1916, emphasized the importance of polymorphonuclear leukocytes in acute glomerulonephritis and expressed the opinion that the number of these cells in the glomerular capillaries affords a distinction between simple inflammatory irritation and true nephritis. In clinical acute glomerulonephritis the polymorphonuclears are often conspicuous and are partly responsible for capillary obstruction when they are distributed among the endothelial cells; but when present alone they rarely cause permanent capillary obstruction since they do not become attached to the capillary wall.

In the subclinical forms of glomerulonephritis which are discussed in this paper, the polymorphonuclears are sometimes present in considerable numbers but usually they are inconspicuous. In estimating the degree of endothelial proliferation these leukocytes were, of course, not enumerated. The leukocytes probably accumulate in the capillaries because the capillary walls have been injured and they may also be held mechanically in capillaries that are partly obstructed by endothelium.

### *Focal Glomerulonephritis*

The embolic glomerulonephritis associated with bacterial endocarditis is a well recognized type. Clinically it is characterized chiefly by hematuria; anatomically there are focal lesions usually considered embolic in origin. But in all probability these focal lesions are thrombotic and proliferative in character and not embolic. They are apparently due to the lodgement of bacteria in the capillary tufts but they are not infarcts (Bell, 1932). They occur frequently in the absence of endocarditis.

Aside from this so-called embolic type associated with endocarditis, focal glomerulonephritis is ill-defined both clinically and anatomically. Clinicians often diagnose as focal glomerulonephritis the transitory hematuria that sometimes accompanies tonsillitis and other infections when no hypertension, edema or renal insufficiency develops. Thus Werboff, 1928, speaks of hematuria accompanying tonsillitis and appendicitis as due to focal glomerulonephritis. Baehr's benign hemorrhagic nephritis, 1926, evidently belongs in this category. The underlying pathology of these transitory hematurias is not known with certainty, but it is probably glomerular bleeding from ruptured capillaries.

There is no clinical condition other than transitory bleeding from the parenchyma of the kidney that can be interpreted as focal glomerulonephritis. Postmortem studies show that albuminuria developing during the course of an infection is due to diffuse and not to focal glomerular injury.

Fahr uses "focal glomerulonephritis" in a pathological sense to include thrombosis or necrosis of individual capillary loops. Only a few glomeruli may be involved and only a part of the affected glomerulus is obstructed. Apparently no constant clinical picture is associated with such focal lesions. Fahr believes that focal glomerulonephritis is due to the lodgement of bacteria in the glomerulus, while diffuse lesions are caused by soluble toxins.

In this series of 865 cases capillary thromboses were rarely seen except in association with endocarditis. Occasionally a few glomeruli show Grade 1 or 2 glomerulitis of a diffuse type when all the others are normal, and usually there are some normal glomeruli when the great majority show glomerulitis. Even in clinical acute glomerulonephritis one may find a few normal glomeruli. A glomerulitis may be focal in the sense that only a small proportion of the glomeruli are involved.

### *The Significance of Endothelial Proliferation*

It is concluded from the foregoing studies that endothelial patterns 0 and + are normal and that the 0 type occurs much oftener in the first than in subsequent decades. A large variety of infectious and toxic processes irritate the glomerular capillaries and cause an increase in the number and size of the endothelial cells. The most pronounced endothelial reactions result from severe streptococcic in-



fections, notably subacute bacterial endocarditis and puerperal sepsis. In these infections the endothelial reaction often approaches and sometimes reaches the intensity that is found in clinical acute glomerulonephritis. It appears that a wide variety of irritants produce glomerulitis of a subclinical type and it is only when a definite capillary obstruction is produced that the clinical symptoms of acute glomerulonephritis develop.

Subclinical glomerulitis differs from clinical acute glomerulonephritis only in the extent of the endothelial proliferation. The fundamental pathological reaction is endothelial proliferation in both conditions; and it seems justified, therefore, to consider subclinical glomerulitis as an early stage of clinical glomerulonephritis. The numerous transitions between the two diseases and the similar etiology also support this interpretation. Cases of acute glomerulonephritis that terminate in complete healing may possibly resemble the severe subclinical forms more than they resemble the fatal acute cases.

*The Nature of the Endothelial Reaction:* The usual interpretation of the endothelial reaction is that it is a proliferative inflammation, i.e. the increase in the number of cells is due to division of preexistent endothelial cells. It is recognized that the endothelial nuclei become larger and that the amount of cytoplasm about them increases greatly. The objection to this interpretation is that no mitoses are to be seen in the endothelial cells. Numerous investigators have confirmed the absence of mitoses. In this study of subclinical glomerulitis no mitoses were seen. One is therefore forced to conclude that if cell division actually occurs it is largely of the amitotic type.

Another theory that merits consideration is that the increase of cells is due to the lodgement of mononuclear leukocytes in the capillaries. It is difficult to distinguish mononuclear leukocytes from endothelial cells unless the former lie free in the lumen of the capillary. In Figures 5 and 6 there are some cells that are obviously mononuclear leukocytes and there are others that may belong to this group. In fact the study of glomerulitis of Grades 1 and 2 brings out considerable evidence that at least some of the increase of intracapillary cells is due to the lodgement of mononuclear leukocytes.

A third theory suggested by Van Waveren is that the endothelial

cells increase only in size and not in number. He explains the appearances of glomerulitis, which I have described, as due to contraction of the capillaries. He is also inclined to believe that true glomerulonephritis may be explained similarly as merely an increase in size of endothelial cells. The drawings (Figs. 3-8) were all made at the same magnification and most of the capillaries are distended, except in Figure 4. It seems incredible that these appearances could all be due merely to increase in the size of the endothelial cells.

We may conclude that glomerulitis is due in part to enlargement of the preexistent endothelial cells and in part to lodgement of mononuclear leukocytes, but endothelial proliferation is probably the most important feature of the reaction.

To what extent is glomerulitis a reversible process? No definite information is available on this problem. On theoretical grounds we may believe it reversible until an intensity is attained that results in the formation of hyaline intracapillary fibers between the cells. The formation of fibers leads to fixation of the cells and permanent obliteration of the capillary. The presence of intracapillary fibers may be used to distinguish the clinical from the subclinical stage of glomerulitis.

If one accepts the theory I have sought to establish in this paper that subclinical glomerulitis differs from clinical glomerulonephritis only in intensity, a broader approach to the etiology of glomerulonephritis is available. A large group of infectious and toxic processes is concerned in the etiology of the disease. The glomerular capillaries are injured probably by various toxic substances. Sensitization to bacterial or other protein may play an important rôle, but it is unnecessary to assume that sensitization is essential in the development of the lesion. Masugi, 1933, has shown that glomerulonephritis develops readily in a sensitized animal when the antigen is injected into the renal artery, but this experiment is about the same as the Arthus phenomenon, and is not duplicated in the clinical development of nephritis. The cases of acute glomerulonephritis that develop within a week after the onset of an acute infection are not easily explained as a result of hypersensitiveness. A widespread sensitization to bacterial protein must be assumed if one is to explain subclinical glomerulitis on this basis.

## SUMMARY AND CONCLUSIONS

A microscopic study of the kidneys was made in 107 cases of death from accidental causes, in 194 cases of death from non-infectious diseases, and in 564 cases of death from various infectious processes.

In the 107 normals the glomerular epithelial cells definitely outnumbered the endothelial in 84.1 per cent, the endothelial outnumbered the epithelial cells in only 1 instance (0.9 per cent), and the two types of cells were approximately equal in number in 15 per cent. It was concluded that a definite preponderance of endothelial over epithelial cells represents a glomerulitis.

A Grade 1 glomerulitis was found in 14 per cent of non-infectious processes.

In lobar pneumonia glomerulitis was found in only 18.8 per cent, but in the other infectious groups it varied from 37.5 to 78.9 per cent.

The highest incidence of glomerulitis was found in puerperal sepsis (52.4 per cent) and subacute bacterial endocarditis (78.9 per cent).

It is evident that a variety of toxic substances, especially those derived from streptococci, may irritate the glomerular capillaries and produce an increase of endothelial cells.

In a Grade 2 glomerulitis the glomerular capillaries are filled with cells and occasionally a few intracapillary fibers are present. The distinction from clinical glomerulonephritis is somewhat arbitrary.

The glomerulitis is probably due chiefly to endothelial proliferation, but the lodgement of mononuclear leukocytes in the capillaries seems to play a rôle of some importance.

There is no relation between the presence or the amount of albumin in the urine and the degree of endothelial proliferation.

There is no anatomical basis for a diagnosis of focal glomerulonephritis except in instances of transitory glomerular bleeding not associated with symptoms of nephritis, and in cases of bacterial endocarditis.

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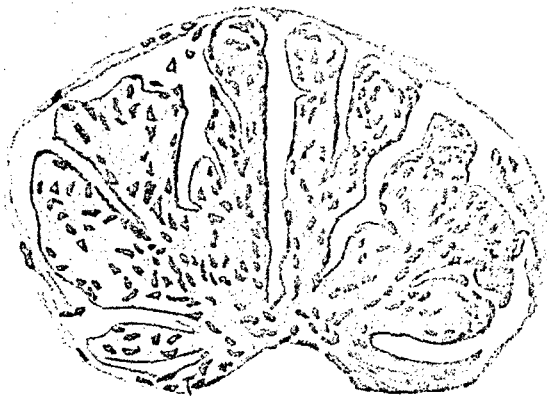
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## DESCRIPTION OF PLATES

### PLATE 129

- FIG. 1. Glomerulus from chronic glomerulonephritis. This is a stage just preliminary to hyaline degeneration. The shrinkage of the lobules accentuates the interlobular septa. Drawing. Low magnification.
- FIG. 2. Glomerulus from chronic glomerulonephritis. The shrinkage is not so great as in Fig. 1, and secondary lobules are more distinct. Drawing. Low magnification.



I



2

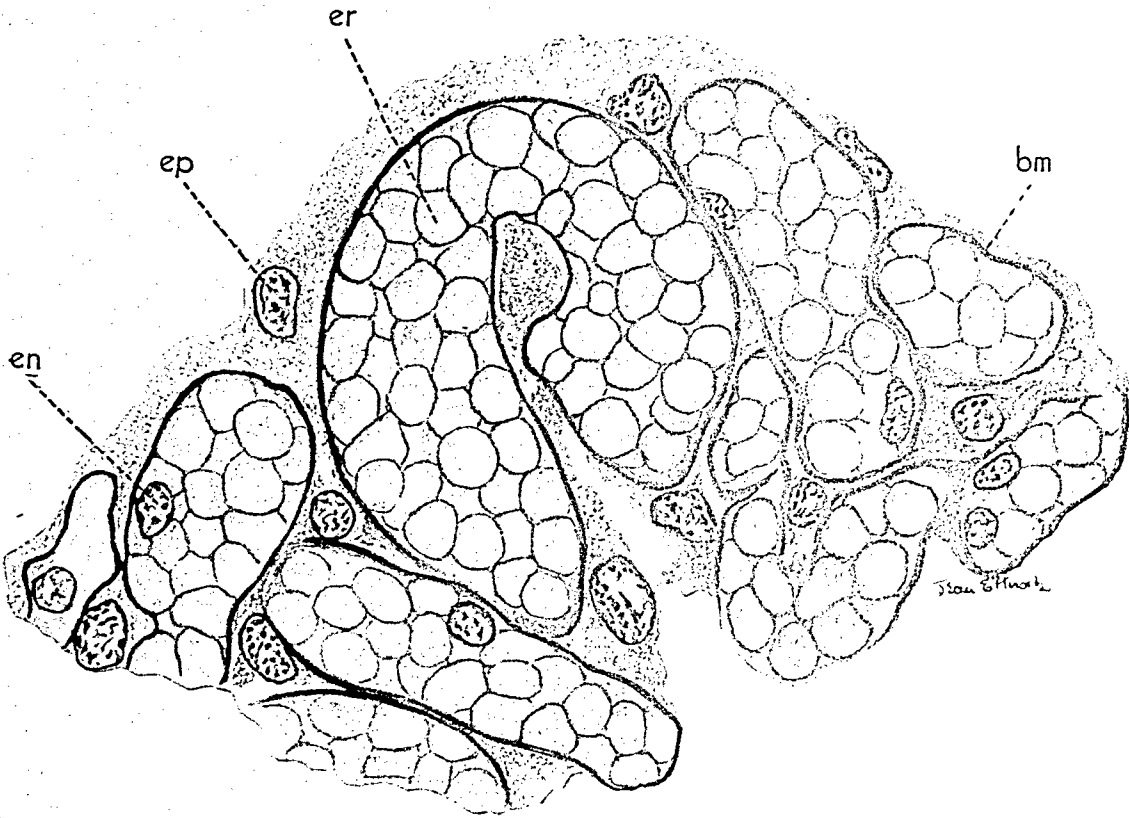
PLATE 130

FIG. 3. Lobule of a glomerulus showing the normal, Grade 0, endothelial pattern. The capillaries are distended. Note that epithelial outnumber the endothelial nuclei. From a case of influenzal pneumonia.

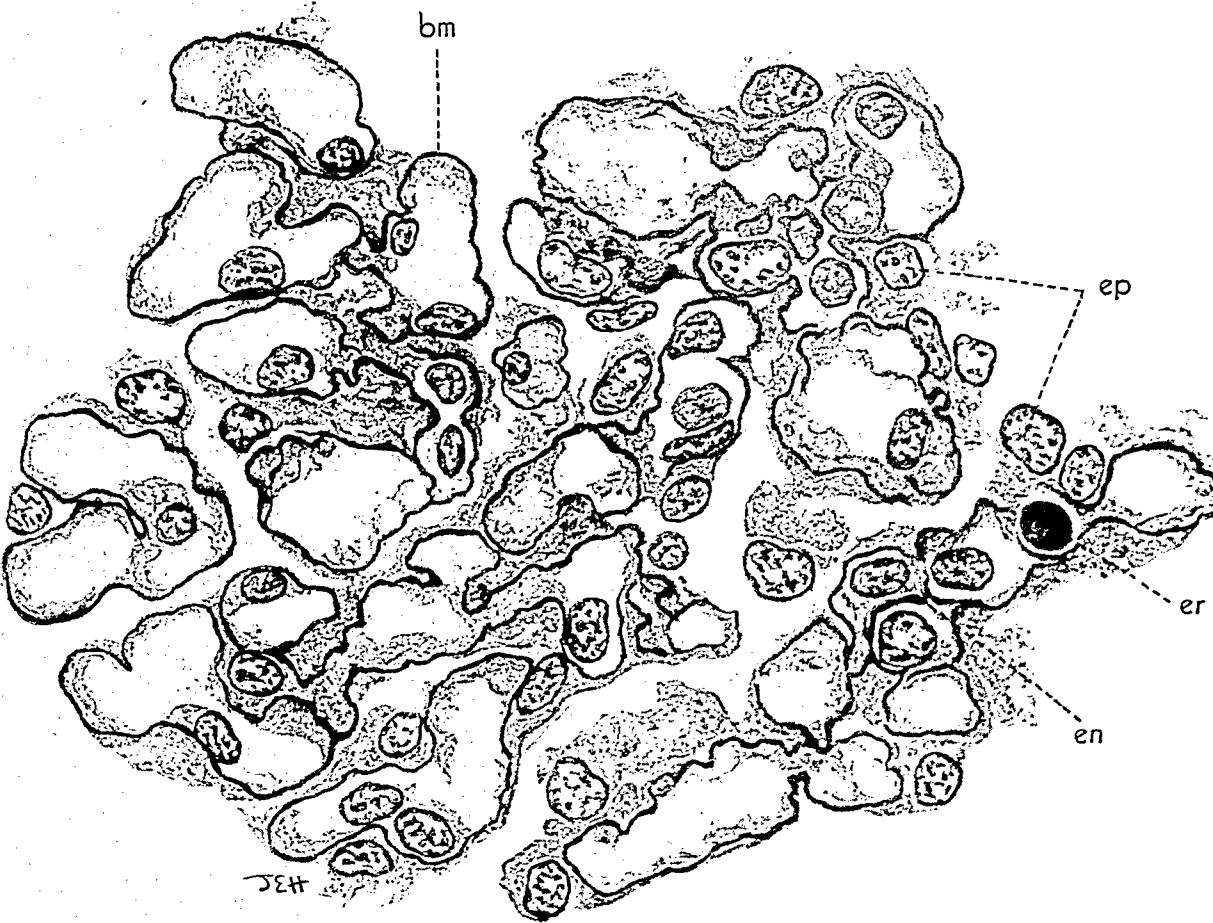
bm = basement membrane of capillary; en = endothelial nucleus; ep = epithelial nucleus; er = erythrocyte. Mallory-Heidenhain stain. Drawing  $\times 1200$ .

FIG. 4. Lobule of a glomerulus showing the normal, Grade +, endothelial pattern. Note that the endothelial and epithelial nuclei are approximately equal in number. The capillaries are empty of erythrocytes but are not collapsed. The basement membrane is somewhat wavy because of the absence of distention. From a case of puerperal septicemia.

Lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing  $\times 1200$ .



3



4



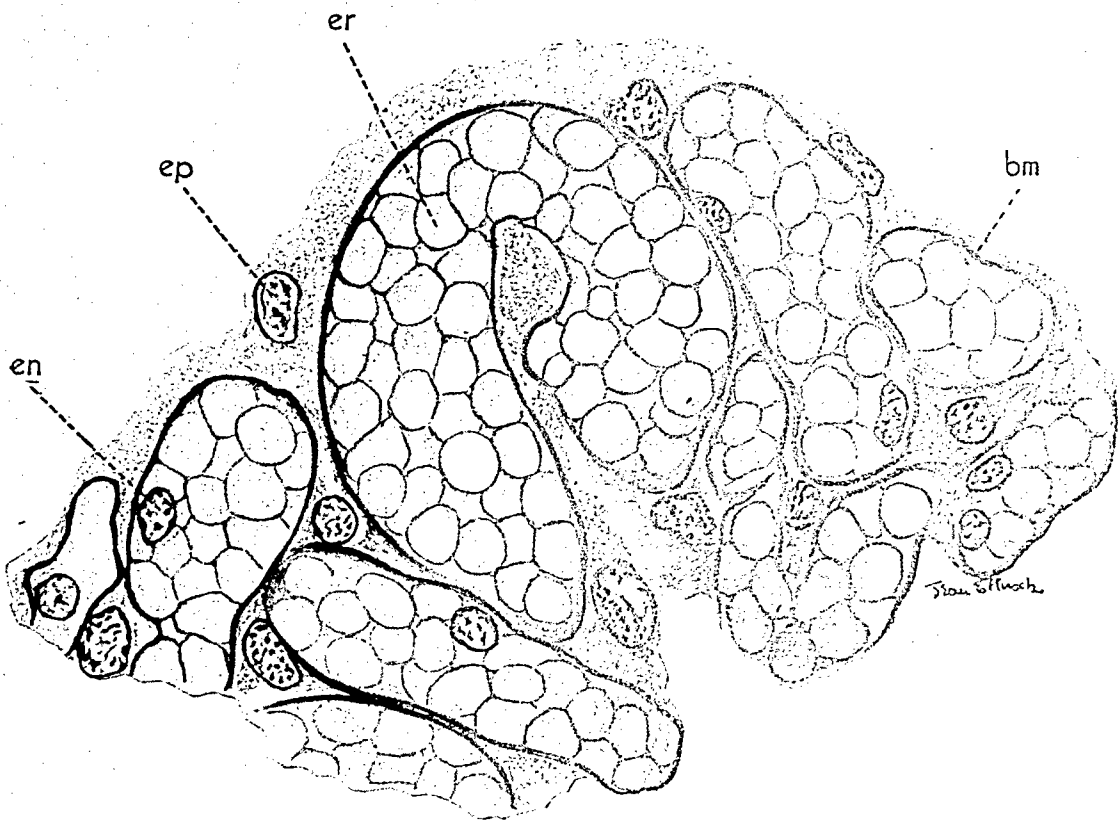
PLATE 130

FIG. 3. Lobule of a glomerulus showing the normal, Grade 0, endothelial pattern. The capillaries are distended. Note that epithelial outnumber the endothelial nuclei. From a case of influenzal pneumonia.

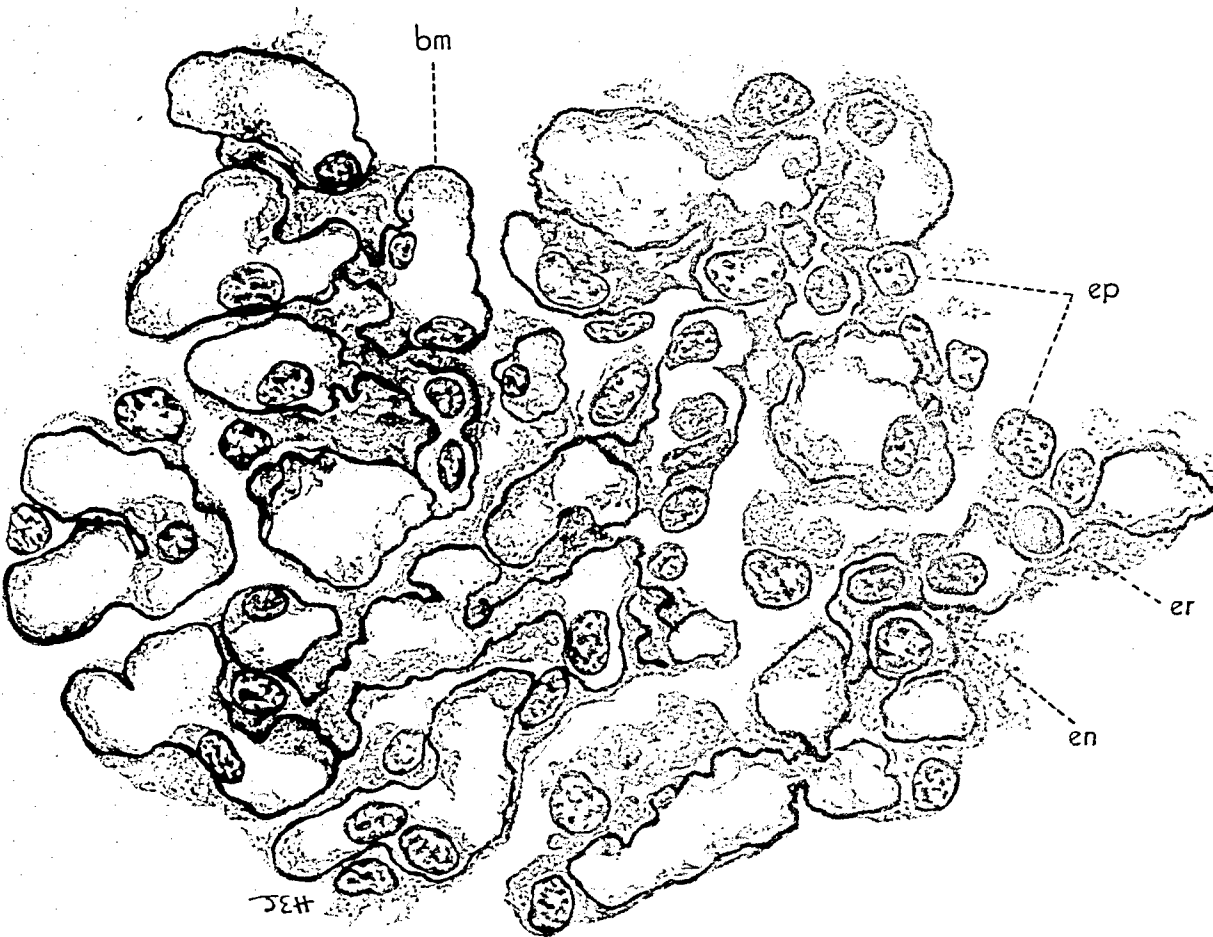
bm = basement membrane of capillary; en = endothelial nucleus; ep = epithelial nucleus; er = erythrocyte. Mallory-Heidenhain stain. Drawing  $\times 1200$ .

FIG. 4. Lobule of a glomerulus showing the normal, Grade +, endothelial pattern. Note that the endothelial and epithelial nuclei are approximately equal in number. The capillaries are empty of erythrocytes but are not collapsed. The basement membrane is somewhat wavy because of the absence of distention. From a case of puerperal septicemia.

Lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing  $\times 1200$ .



3



4

Early Stages of Glomerulonephritis

PLATE 131

FIG. 5. Lobule from a glomerulus showing Grade 1 glomerulitis. The endothelial cells definitely outnumber the epithelial and have produced a partial capillary obstruction. Nearly all the cells within the capillaries appear to be of endothelial origin, but one definite mononuclear leukocyte is shown. From a case of septicemia.

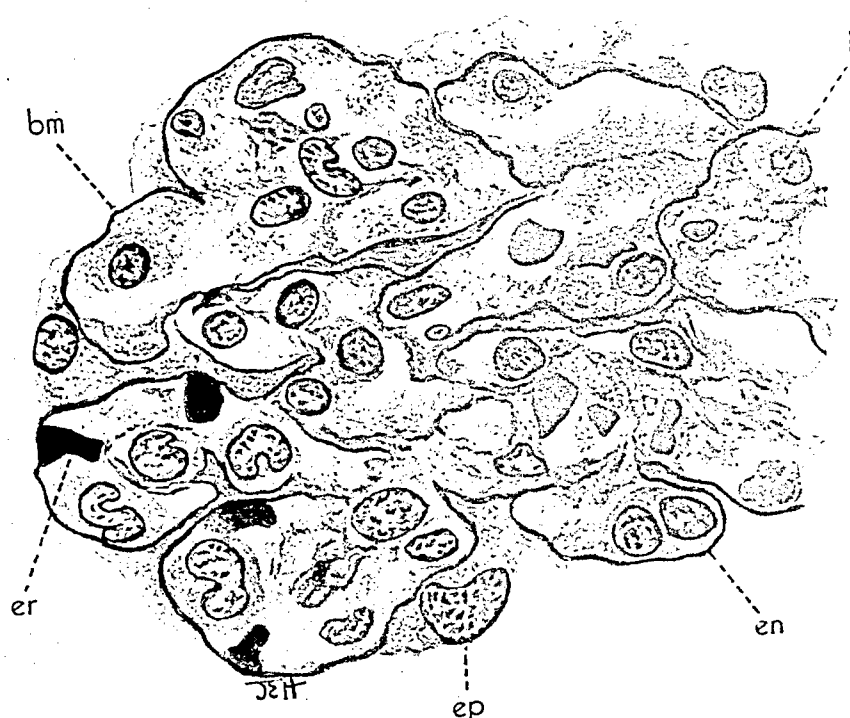
l = mononuclear leukocyte. Other lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing  $\times 1200$ .

FIG. 6. Lobule from a glomerulus showing Grade 2 glomerulitis. The capillaries are somewhat more closely packed with cells than in Fig. 5. A few erythrocytes are seen which are distorted by pressure. An occasional definite mononuclear leukocyte is seen, and the cells with indented nuclei may be leukocytes. From a case of septicemia.

l = mononuclear leukocyte. Other lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing  $\times 1200$ .



5



6

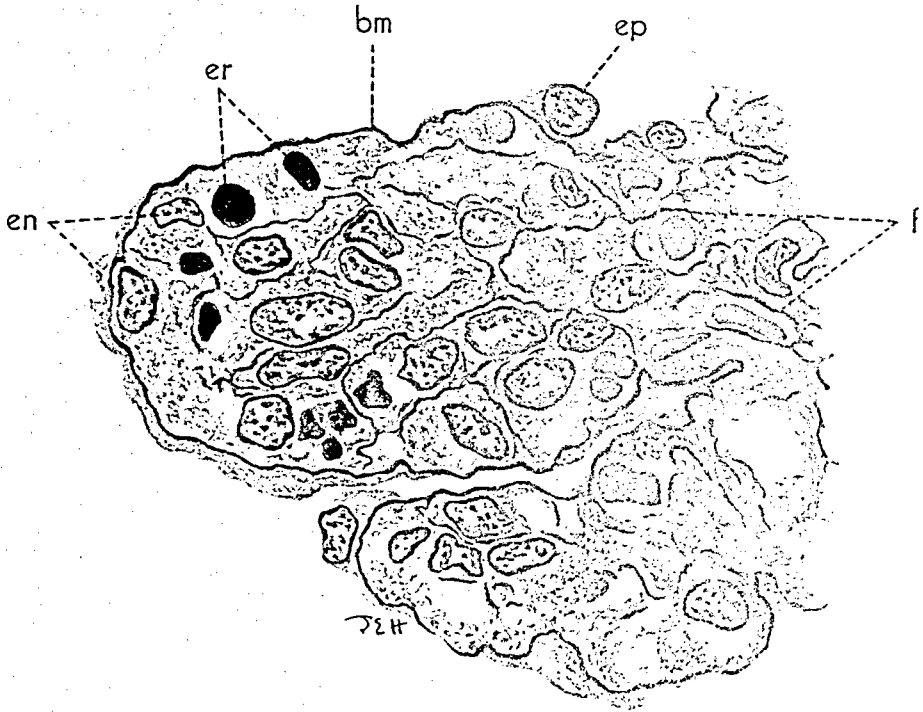
PLATE 132

FIG. 7. Lobule from a glomerulus showing the Grade 3 endothelial pattern. This is clinical acute glomerulonephritis in an early stage. Death from an associated infection. The capillaries are greatly distended.

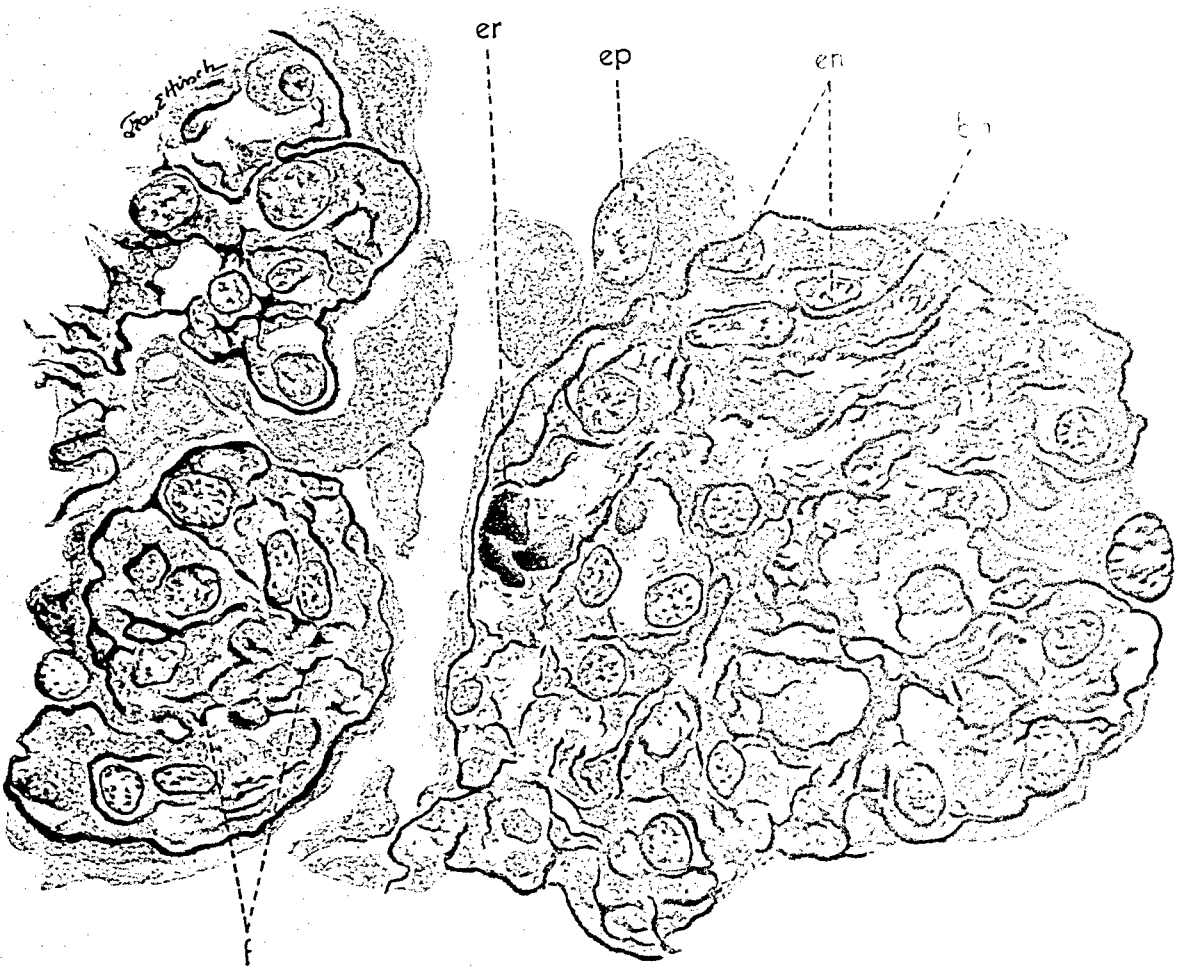
Note the intracapillary fibers, f. Other lettering as in Fig. 3. Mallory-Heidenhain stain. Drawing  $\times 1200$ .

FIG. 8. Lobules of glomerulus from a typical case of acute glomerulonephritis, more advanced than in Fig. 7. Death from uremia.

Note numerous intracapillary fibers, f. Other lettering as in Fig. 3. Mallory-Heidenhain stain  $\times 1200$ .



7



8

Early Stages of Glomerulonephritis



# THE INTERPLAY OF THE CELLS OF THE HEMATOPOIETIC TISSUES IN RABBITS INFECTED EXPERIMENTALLY WITH THE TUBERCLE BACILLUS\*

## THE ORIGIN OF THE MONOCYTE CONSIDERED \*

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The hematopoietic tissues are in essence mobile, with quasi-fixed portions which serve to manufacture the various cell types that eventually enter into and determine the composition of the circulating blood (the mobilized portion of the system). In postembryonic life specialization of blood cell production occurs in various locations in the body, and the products of widely separated regions of hematopoiesis are brought together through the lymph- and blood-vascular systems. The specialization of blood cell production has led to the designation of the following "systems" of hematopoiesis: (1) myeloid system (bone marrow); (2) lymphoid system (lymph nodes, spleen and scattered lymphoid aggregations, as in the gut); and (3) reticulo-endothelial system (the system of the cell of many aliases, some of which are histiocyte, monocyte, macrophage, endothelial leukocyte, resting wandering cell, and so on). Aschoff<sup>1</sup> limits this system to the spleen, liver, lymphoid tissue and bone marrow, while by others it is extended to include the whole lymph- and blood-vascular system. While the "pigeon-holing" of the blood-forming apparatus into "system" of "fixed" tissue serves a useful purpose, the fact that the hematopoietic tissue is essentially mobile should not be forgotten.

It is apparent that nature has evolved the composition of the blood so that under normal physiological conditions a functional interplay of the different cell types can occur. With the advent of pathological processes extensive alterations may occur in the cellular composition of the blood. The variations from normal depend in large part on the type, extent and gravity of the damage produced by a viable (bacterium, malaria plasmodium, trichina, and so on) or by a chemical (croton oil, and so on) agent. Seldom, if ever, is the alteration confined to one cell type in the blood. And behind the

\* Received for publication March, 30, 1936.



variations in the circulating blood changes occur in the factories that produce the blood cells. It becomes obvious then that for a sufficiently broad and inclusive comprehension of the functional interplay in the hematological response to any pathological process representative samples from all of the "systems" of hematopoiesis should be carefully studied. Such examinations should be made serially during the evolution of the pathological process under consideration to determine the possible significance of single observations to the picture as a whole.

It is the purpose of this report to present the conditions found in the hematopoietic tissues during the evolution of the type of tuberculous infection that invariably caused death and to discuss the cellular interplay observed. For ease of presentation the changes found will be given under the headings of "systems."

#### METHODS AND MATERIAL

We have used the rabbit as the animal for our experiments. During the study, which has extended over a 5 year period, over 100 animals have been used. In those experiments where the different stages of tuberculosis were to be studied, groups of 12 animals were inoculated intravenously with the same dosage of either virulent avian or bovine tubercle bacillus on the same date. The rabbits were then sacrificed in pairs at 24 hours, 5, 10, 14 and 21 days after inoculation, one pair of animals always being left to succumb to the infection. Twelve rabbits were inoculated intravenously with a recently isolated and highly pathogenic strain of *Staphylococcus aureus*. Of these, 2 were sacrificed at the end of 24 hours and the remainder were allowed to die of the infection. All but 2 of the animals died within a week. One lived 16, and another 20 days.

For the data on the changes in the hematopoietic tissues in vaccinated rabbits we used material from other experiments in which over 50 rabbits were used. These animals were vaccinated in groups of 6 to 12 by inoculating human tubercle bacilli (H37) subcutaneously, or by the intravenous injection either of living BCG or of heat-killed virulent bovine bacilli. Two to 6 months were allowed to elapse following the last dosage of vaccine before living virulent bovine bacilli were injected intravenously. Following the injection of the living virulent bacilli the same procedure was followed as for the non-vaccinated rabbits.

The dosage of tubercle bacilli ranged from 0.01 to 5 mg. The photomicrographs shown in the plates were from experiments in which the larger dosage of bacilli was used. The same general changes occurred when smaller amounts of bacilli were used, but the intense acute response of the hematopoietic tissue to the larger dosage rendered tissues from such animals superior for purposes of illustration.

Frequent leukocytic counts were done on all of the animals, both prior to and after inoculation, as can be seen from the tables in the text. The animals chosen to show the leukocytic picture were representative of the groups from which they were taken and had all succumbed to the infection.

Complete autopsies were done on all of the animals used in the experiments and histological preparations were made on all organs. As representative of the hematopoietic tissue, samples from the following were routinely examined: (1) myelogenous system (femur bone marrow); (2) lymphoid system (spleen, mesenteric lymph nodes, and vermiform appendix); and (3) reticulo-endothelial system (the liver, since it is supposed to be the most important portion of this system, aside from the "reticular cells" of the myelogenous and lymphoid tissue).

## RESULTS OBTAINED

### *Myelogenous System (Femur Bone Marrow)*

The cellularity of the femur marrow varies to a considerable extent in different rabbits under normal conditions so that a fixed normal cannot be established. In general, the central portion of the marrow is composed largely of fat cells with the portion adjacent to the bone being the active blood-forming part. The width of this peripheral rim shows considerable individual variation. Occasional, small isolated islands of hematopoietic tissue (Fig. 1) are present in the central area. Mitoses are occasionally found in the peripheral area but have not been observed in the central portion.

At the end of 5 days after tubercle bacilli had been inoculated an increase of hematopoiesis (Fig. 2) was demonstrable in the central portion of the bone marrow. Here the main increase was in immature undifferentiated cells, in maturing myelocytes and in megakaryocytes. Occasional mitotic figures were present in the undifferentiated cells. Mitoses were more frequent than normal in the

peripheral zone where the general cellular picture was similar to that in the central portion of the marrow. Mature segmented neutrophils were scarce.

At 10 days (Fig. 3) a considerable hyperplasia in the central portion of the marrow was evident. The marrow tissue showed widely separated fat cells throughout. The majority of the cellular increase was composed of immature undifferentiated cells. Mitotic figures were easily found in these cells throughout the marrow. Mature cells of both the myelocytic and the megakaryocytic series were proportionally reduced. No definite tubercles were present, although tubercle bacilli in individual monocytes could be demonstrated. The capillary endothelium appeared normal.

At 14 days (Fig. 4) a still greater hyperplasia had occurred. The fat cells were very widely separated, as can be seen in the photograph where these cells appear as clear spherical spaces. Mitoses were still abundant and undifferentiated cells predominated. Megakaryocytes of the large mammalian type, some of which were emigrating (Fig. 5), were definitely increased in number. Maturing myelocytes were more abundant than at 10 days but segmented neutrophils were scarce. There was no line of demarcation between the central and peripheral portions of the marrow. Isolated, well defined monocytic tubercles were in evidence. They were more numerous in the peripheral portion of the marrow, as shown in the illustration. These tubercles did not appear to be connected definitely with the blood vessels. They were composed almost wholly of monocytes which were in a good state of preservation. Mitotic figures in the tubercles were found (Fig. 6, in the right upper quadrant) but they were rare. The hematopoietic tissue surrounding the tubercles was in an active state of proliferation, mitoses being frequently encountered.

At 3 weeks (Fig. 7) in avian tuberculous infection a most extensive tuberculosis of the marrow was present. Such extensive involvement was not observed with the bovine type of organism. The tuberculous area of involvement had the appearance of a large continuous sheet of monocytes, the picture of small isolated tubercles, as seen in Figure 4, having largely disappeared. Tubercle bacilli were numerous. Mitotic figures in this area were rare. Giant cells (tuberculous) were rare. Hematopoiesis was active outside of the tuberculous areas. In the non-tuberculous areas the cellular picture was essen-

tially the same as at 14 days. Where the tuberculous tissue and the hyperplastic marrow tissue joined (Fig. 8) the monocytes were in a good state of preservation. Deeper in the tuberculous area (Fig. 9), toward the center of the marrow, there were areas where considerable necrosis of monocytes had occurred. In many of these latter areas a slight to moderate invasion of neutrophiles (the irregular deeper staining nuclear structures seen in Fig. 9) had taken place.

This completes the essential changes usually found in the marrow in the acute tuberculous infection. The large majority of rabbits died of the infection, whether of avian or bovine type, within 4 to 5 weeks. The marrow picture did not differ essentially in these animals from the condition seen at the end of 3 weeks. An occasional rabbit inoculated with the bovine tubercle bacillus lived for 2 months. Rarely such an animal might show tuberculous foci with caseous centers (Fig. 12). This condition was more often found when rabbits were given a smaller dosage of bovine bacilli and lived for several months, although even here such findings were infrequent. It was of interest, however, that this type of lesion, which was so frequently seen in the lung and kidney, could also occur within the bone marrow.

In *vaccinated* rabbits inoculated with 1 mg. of virulent avian or bovine tubercle bacilli intravenously, the marrow changes were the same within the 3 week period, as observed in non-vaccinated rabbits, except that the hyperplasia appeared to be more rapid. Some of the vaccinated reinfected rabbits lived for several months. The condition of the marrow at the time of death of these reinfected, chronically ill animals was of interest. It was uniformly devoid of fat and congested (Fig. 10). It was much less cellular than at the height of hyperplasia (2 to 3 weeks), but the hematopoietic tissue was still notably active in the production of cells, as shown from the frequency of mitotic figures. In Figure 11 six mitotic figures could be determined with certainty under the microscope. In these marrows tuberculous lesions were rare. When present (Fig. 10), they showed circumscribed areas of monocytes, occasional Langhans' giant cells, and moderate to intense lymphocytic infiltration. These marrows showed considerable variation in their megakaryocytic content. In some, megakaryocytes were abundant; in others, they were not increased over normal. The outstanding feature of these mar-

rows was the preponderance, in all specimens examined, of myelocytes and maturing neutrophils.

In the study of all the marrow tissues an impression was gained that the erythrogenic tissue was at times definitely increased. This, however, had to remain only as an impression since the outstanding marrow hyperplasia was on the undifferentiated cell, myelocytic and megakaryocytic side.

While it is the purpose of this paper to present the changes observed in tuberculous infection, it is not amiss to cite briefly changes that occur in another type of infection. In rabbits inoculated intravenously with *Staphylococcus aureus* the marrow changes were quite in contrast with those found in tuberculous infection. The hyperplasia in staphylococcal infection was, from its onset, predominantly in the neutrophilic series. Because of this the cellular pattern of the marrow did not appear so complex. The differences in the two pictures suggested that the basic demand placed on the marrow was much more unicellular in type in staphylococcal than in tuberculous infection.

#### *Reticulo-endothelial System (Liver)*

This organ was chosen to represent the so-called reticulo-endothelial system because, in postembryonic life, it is normally free from the other types of hematopoietic tissues. In the normal rabbit liver the reticulo-endothelium (Küpferr cells) can be fairly easily recognized. It is not abundant.

It was found, as is commonly known, that there was a distinct difference in the extent of tuberculous involvement of the liver in bovine and avian tuberculous infection. The avian tubercle bacillus caused much more extensive pathological lesions. The type of cellular response in the individual tuberculous lesion was essentially the same in both types of infection. The accumulations of cells, whether few or many, large or small, occurred almost wholly within the liver capillaries. With the avian tubercle bacillus the greatest involvement was at the period when the animals succumbed, whereas with the bovine type the amount of tuberculosis was usually considerably less when the animals died than at an earlier period.

At 5 days (Fig. 13) the tuberculous lesions consisted of small intracapillary accumulations of mononuclear cells. These lesions were

scattered throughout the organ. The intervening capillaries appeared normal without any evidence of increase or of mitosis in the Küpffer cells. An occasional mitotic figure was found in the tuberculous foci.

At 10 days (Fig. 14) the lesions were more numerous and had the typical appearance of monocytic tubercles. In such foci mitoses were rare. Here again the reticulo-endothelial system away from the focal lesions showed no evidence of hyperplasia.

From 10 days onward in the avian tuberculous infection the volume and number of lesions increased, so that when the animals died at 4 to 5 weeks, large areas composed of capillaries tremendously distended with monocytes were found. The columns of liver parenchyma were compressed between the distended capillaries. At 3 weeks there were many areas of uninvolved liver tissue which showed little, if any, evidence of hyperplasia of the Küpffer cells. In Figure 15 the intracapillary nature of the monocytic tubercle is shown. It is also evident that in the sinusoids adjacent to but separated from the tuberculous lesion there is no evidence of hyperplasia of the reticulo-endothelial system. Some necrosis of monocytes and a beginning invasion of neutrophils are to be seen in Figure 15. In such lesions tubercle bacilli are abundant.

It is apparent that if the reticulo-endothelial system of the liver does participate in the reaction to the tuberculous infection it does so only in those areas in which the tubercle bacilli lodge. No general hyperplasia of the system such as was found in the myelogenous system had occurred.

In *vaccinated* rabbits reinfected intravenously with avian tubercle bacilli the pathology in the liver differed in some respects from that observed in non-vaccinated animals. The location of the reaction was the same. In the vaccinated animals there often occurred as great a reaction in a week as was found in the non-vaccinated in 2 to 3 weeks. In the vaccinated animals that lived for several months after the introduction of the reinfection a considerable variety of lesions was found. In such animals the major part of the organ was free from infection. The varieties of lesion found were: monocytic tubercles, monocytic tubercles having caseous centers with or without giant cells, isolated foreign body giant cells, small abscesses, foci of lymphocytes, and fibrous scars with or without lymphocytic infiltration. Thus it is seen that vaccination has enabled the animal

to react to the tuberculous infection in a way differing from a first infection.

In the *staphylococcal infection* lesions in the liver were rarely found. Occasional Küpffer cells or neutrophiles might contain the bacteria. When definite accumulations of cells did occur they were predominantly neutrophilic in type.

### *Lymphoid System (Spleen)*

The lesions in the spleen are perhaps logically followed by considering the flow of blood through the organ. The arterial system has intimately associated with it closely packed aggregates of small round cells commonly regarded as lymphocytes. These aggregations form a distinctive feature of the architecture of the organ and are spoken of as malpighian corpuscles or germinal centers. The arterial branches pass outward through the germinal centers further to subdivide in the extracorpuseular splenic tissue — the pulp. This portion of the spleen has an exceedingly complex and abundant capillary network lined by endothelium. Between the capillaries is a variable number of round cells which are usually larger than the small ones present in the germinal centers. On the distal side of the pulp the capillaries unite to form the venous sinuses which in turn unite to form branches of the splenic vein. In the venous sinuses the endothelial lining is especially prominent. The terms "germinal center," "pulp," and "sinuses," will be used to designate the locations of the cellular changes observed within the organ.

The germinal center of the normal rabbit spleen (Fig. 18) varies somewhat in size but is always composed of a rather dense mass of small round cells surrounded by a "collar" of larger and paler staining cells. The prominence of the "collar" varies considerably in different animals. The pulp cells resemble those seen in the "collar" of the germinal center. The number of these cells varies considerably. The endothelium of the sinusoids is usually composed of a single layer of flattened cuboidal cells which resemble the cells of the pulp in staining reaction. The remainder of the cellular content of the organ is largely that of the circulating blood.

In tuberculosis the spleen becomes larger than normal (Fig. 16). The degree of enlargement varies considerably. It is especially great in the avian type of infection (Fig. 17) in the rabbit where the organ may become 3 inches long, 1 inch wide and one-half inch thick.

The changes in the spleen following the injection of tubercle bacilli were of the same general character whether the bovine or avian tubercle bacillus was used. Tuberculous involvement was however always much more extensive in the avian type of infection. Within 5 days after inoculation (Fig. 20) hyperplasia of the germinal center had begun, as is evident from the size of the unit and of the "collar." The proportion of the larger cells was increased and mitotic figures were numerous (Fig. 21). There did not seem to be a distinct increase of cells in the pulp or the sinusoids. Mitoses in these latter regions were rare and tubercles were absent. Tubercle bacilli could be demonstrated after careful search in single cells of monocytic type.

Ten days after infection the changes noted above in the germinal center were simply more pronounced (Fig. 22). The small, densely staining type of cell was proportionally reduced. Mitotic figures were numerous (Fig. 23), suggesting a malignant splenic tumor. The cellular content of the pulp was definitely increased and occasional mitotic figures were present. The endothelium of the sinusoids did not appear to have participated to any appreciable extent in the hyperplasia. Among the circulating blood cells megakaryocytes, which in all probability had migrated from the bone marrow, were occasionally seen. An occasional monocytic tubercle was found in the pulp. Tubercle bacilli were easily demonstrable in single cells of monocytic type as well as in the definite tubercles.

The picture seen at 14 days differed from that at 10 days in that typical monocytic tubercles were abundant, some of them being of considerable size. These tubercles were especially prominent at the periphery of the hyperplastic germinal center (Fig. 24). Tubercles were also scattered irregularly through the pulp. It seems as if the large pale staining monocytes of the tubercles had arisen from the cells seen in the "collar" of the germinal center. It was usual to find an admixture of small, deeply staining cells, medium sized, lighter staining cells and the large pale monocytes of the tubercle in the "collar" (Fig. 25). The cellularity of the whole splenic structure was greatly increased. Mitotic figures were found fairly easily, even in monocytes lying free in the blood within the sinuses (Fig. 27). A rare mitotic figure was observed in cells that may have been the endothelial cells of the sinusoids. Tubercle bacilli were abundant, more so in the avian than in the bovine type of infection.



From the 2nd week onward the further changes in the spleen were found chiefly in the tuberculous foci. In the avian type of infection the organ was largely converted into "sheets" of monocytes (Fig. 17, light staining areas) with many of the germinal centers being recognizable only from the presence of the artery and small clumps of small lymphoid cells (Fig. 26). In large areas the monocytes appeared well preserved. There were foci in which these cells had necrosed and in such areas invasion of neutrophils was frequently found (Fig. 28). In a few areas the neutrophils had also necrosed, and where this had occurred the typical appearance of caseation was seen. Foreign body giant cells were often abundant in the later stages, especially in the bovine type of infection where the spleen was not so heavily involved. While the splenic tissue remained hyperplastic throughout the course of the disease, mitoses were less easily found at the time of death. Monocytes in mitosis were occasionally found in the tuberculous lesions, and even on rare occasions in giant cells.

In some of the spleens megakaryocytes were quite numerous. There were also the other cells of the myelogenous tissue present. No conclusive evidence could be found that myelogenous tissue was being produced in the spleen.

The reaction that occurred in the spleen in *vaccinated* animals reinfected intravenously with avian tubercle bacilli differed in many respects from that noted in a first infection. Individual tuberculous monocytes and monocytic tubercles were present in all portions of the organ within 5 days and were often more abundant at this early date than at 2 weeks in the animals with a first infection. In the vaccinated animals that survived reinfection for several months the spleens were always found to be within normal size and in some instances appeared smaller than normal. Microscopic examination of such spleens revealed but little evidence of tuberculosis. There were at times small monocytic tubercles or foreign body giant cells in the pulp or in the germinal centers. The germinal centers were consistently smaller and less cellular than normal. No mitotic figures could be found. The cells were largely of the small lymphocytic, with a rare cell of the monocytic type. In the pulp there was evidence of increased reticulum, relatively few pulp cells and considerable amounts of blood pigment in and between the capillaries. In many instances the pigment accumulation was so

large as to distend the capillaries. Pigment-laden monocytes were frequently seen. The endothelial lining of the capillaries and large sinusoids appeared normal. Thus it is seen that through vaccination the animals were enabled to heal, by resolution, the large portion of the tuberculosis of the spleen. It is also quite apparent that with the development of chronic progressive tuberculosis in the kidneys, joints and lungs, the demand for cells of the type manufactured in the spleen became distinctly less than during the earlier stages of the disease. This phenomenon is of real importance since it reflects a mechanism within the different portions of the hemato-poietic tissues which responds with delicacy to the demand for cells.

The intense hyperplasia of the splenic tissue and the extensive tuberculous lesions were in great contrast with what happened in *staphylococcal infection*. In the latter the organ was not enlarged if the animals died within a week. Microscopic study showed that there was no evidence of hyperplasia (Fig. 19). If animals lived more than a week the spleen might be slightly enlarged and a suggestion of mild hyperplasia might be found. The contrast between the spleen in tuberculous and in staphylococcal infection suggested a fundamental difference in the demands placed on the organ in the two types of infection. There was also a suggestion that if an animal had sufficient resistance to survive a staphylococcal infection for a considerable time the splenic tissue was called upon, to a mild degree at least, to participate in the disease process.

### *Lymphoid System (Appendix)*

This organ, because of its abundant lymphoid tissue, was chosen to represent the lymphoid system apart from the spleen. In the rabbit the lymph nodes do not seem to participate as much in the tuberculous process as they do in the guinea pig. This may be due in part, but not entirely, to the use of a different method of inoculation. Mesenteric lymph nodes were routinely studied in the rabbits and considerable alteration from normal was found. It was not clear, however, whether the changes noted were due to the reaction of the essential tissue of the nodes or to the accumulation of cells from the lymph channels that drained the lymphoid tissue of the gut. Tuberculous lesions in the nodes were rare unless lesions were also present in the gut.

The unit of lymphoid tissue in the normal appendix (Fig. 29) is somewhat flask shaped. The portion toward the serosa has a rim of closely packed lymphoid cells, while the central portion is composed of a network of blood and lymph capillaries, between which lymphoid cells are closely congregated (Fig. 32). A rare mitotic figure may be found in the peripheral zone. The portion adjacent to the mucosa shows closely packed lymphoid cells which are smaller than those in the serosal region. The amount of lymphoid tissue in normal animals varies considerably but it is seldom more abundant than in the illustration given (Fig. 29).

Within 5 days after the inoculation of tubercle bacilli the lymphoid tissue showed evidence of beginning hyperplasia. This regenerative activity reached its height in 10 to 14 days. Under low power the volume of lymphoid tissue was found to have become greatly increased (Fig. 30), predominantly so in the serosal portion. Under high power (Fig. 33) the latter area showed the peripheral portion definitely thickened and mitotic figures abundant. The central area was also much more cellular than normal and occasional mitoses were found in this region. A study of the area adjacent to the mucosa showed an apparent increase of cells which were smaller and more typically lymphocytes (Fig. 34) than those in the serosal area. Mitotic figures in this portion were rare. As one followed the tissue structure from the mucosa toward the serosa a fairly sharp dividing line occurred between the size of cells and the frequency of mitotic figures.

The intense hyperplasia of the lymphoid tissue of the appendix apparently was not due to the presence of tuberculous infection in the tissue. Tubercles were extremely rare in both the avian and the bovine type of infection when the rabbits died within 6 weeks. The bacilli in isolated cells, so easily demonstrated in other organs, were not found in this tissue.

Tuberculous lesions of the gut do occur when rabbits infected with bovine tubercle bacilli survive for several months. As a rule the longer the animal lives the more numerous are the lesions. The lesions are limited almost wholly to the appendix and the beginning of the cecum. They are due apparently to the ingestion of tuberculous pus from open, ulcerative pulmonary tuberculosis. The foci are irregularly distributed and always occur in the portion of lymphoid tissue adjacent to the serosa (Fig. 31). They show the same sequence

of events that occurs in tuberculous lesions in other tissues. The early tubercle is composed of well preserved monocytes (Fig. 35). When and if the monocytes undergo necrosis such areas are invaded by neutrophils (Fig. 36) and with the death of the latter cells the typical picture of caseation is produced.

A study of the uninvolved areas of lymphoid tissue in the late stage of tuberculosis was of interest. A comparison of Figures 30 and 31 shows that the serosal portion is less prominent and the mucosal portion is more in evidence in Figure 31. The mucosal area is composed of small typical lymphocytes. The serosal portion is less cellular than normal. Mitotic figures occur in this latter area but they are infrequent.

The reaction of the tissue in *staphylococcal infection* corresponded to that noted under the spleen in the same type of infection. This tissue apparently participated but little in the disease process.

#### *Circulating Blood (Mobilized Hematopoietic Tissue)*

Wide fluctuations in the cellular content of the circulating blood in rabbits are so common that it is necessary to give much greater latitude for the normal picture than in man. The picture varies so much from rabbit to rabbit that it is advisable to use each animal as its own control. Whether this instability of the blood picture is due to a more labile physiological set-up or to the presence of unrecognized natural infections is difficult to determine. Examination of several hundred apparently healthy rabbits has suggested to the authors that the fluctuations found are more likely to be due to an unstable physiological state than to intercurrent infection.

The cell types are the same in the rabbit and in man, although the staining reaction and the granular content of the different leukocytic types are somewhat different. As a rule the lymphocytic content equals or exceeds the neutrophilic. The lymphocytes commonly outnumber the neutrophils at least two to one. The monocytic type of cell may, on occasion, be over 10 per cent but it is usually between 3 and 8 per cent. Basophiles (sometimes called the "x" cells) vary from 2 to 10 per cent but on occasion may be more numerous. Eosinophiles show the lowest percentage of any type of leukocyte. Nucleated red cells may be seen on occasion. The total leukocytic count tends to be quite variable.

The technic used in making the total leukocytic counts was that

commonly employed. For the differential counts 400 leukocytes were counted on blood smears stained with Wright's stain. For the modified Schilling count the neutrophils were divided into seg-

TABLE I

*Rabbit No. 8. 1 mg. Avian Tubercle Bacilli Injected Intravenously*

Leukocytic record						
Date	Total count	Neutrophiles	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%
4/17/33	21,800	17	68	8	3	4
4/18	12,700	24	62	9	2	3
4/19	19,000	20	67	7	1	5
4/20	17,000	15	78	5	1	1
4/21	12,700	40	50	8	0	2
4/22	10,700	26	60	7	3	4
4/23	11,500	20	63	8	3	6
(4/24 1 mg. avian tubercle bacilli intravenously)						
4/24	12,000	33	59	6	1	1
4/25	8,700	33	54	10	2	1
4/26	14,200	35	45	15	1	4
4/27	6,300	23	67	7	1	2
4/28	8,400	40	34	17	2	7
4/29	10,500	23	58	10	4	5
4/30	5,200	20	66	8	2	4
5/1	7,400	28	61	9	1	1
5/2	6,500	28	57	9	3	3
5/3	9,400	19	67	10	2	2
5/4	8,600	16	76	6	1	1
5/5	15,500	9	61	29	0	1
5/6	18,600	14	40	44	0	2
5/7	13,200	18	52	28	1	1
5/8	31,400	23	37	39	0	1
5/9	14,000	17	21	60	0	2
5/10	9,100	18	54	25	0.5	2.5
5/11	8,900	34	40	25	0.5	0.5
5/12	25,300	15	48	35	1	1
5/13	17,700	17	32	48	0.5	2.5
5/14	17,000	27	30	42	0	1
5/15	17,000	29	25	44	0	2
5/16	6,500	32	34	33	0	1
5/17	7,300	32	23	45	0	0
5/18	15,000	26	20	50	1	3
5/19	14,500	36	22	41	0	1
5/20	5,300	50	22	27	1	0
5/21	7,500	29	18	53	0	0
5/22	13,300	46	30	24	0	0
5/23	11,900	47	21	32	0	0
5/24	13,000	51	20	29	0	0
5/25	Dead					

mented and non-segmented forms. In some of the tables the percentage of immature or non-segmented forms is given. In the immature cells we included all forms that could be definitely identified as belonging to the neutrophilic series and that were not segmented.

TABLE II

*Gray Rabbit. 5 mg. Virulent Bovine Tubercle Bacilli Injected Intravenously*

Leukocytic record							
Date	Total count	Neutrophiles	Non-segmented neutrophiles 100 cells counted	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%	%
8/22/33	7,000	46	15	43	6	1.5	3.5
8/23	8,400	30	23	61	4	1	4
8/24	6,900	40	22	49	5	0	6
8/25	6,500	47	17	44	4	1	4
8/26	6,800	34	6	58	5	0	3
8/27	9,800	36	16	53	7	0.5	3.5
8/28	11,700	34	20	48	7	2	9
(8/28 5 mg. virulent bovine tubercle bacilli intravenously)							
8/29	12,900	34	29	52	9	0.5	4.5
8/30	10,700	37	30	51	9	0	3
8/31	8,300	40	18	50	7	0.5	2.5
9/2	9,700	53	32	36	9	0	2
9/4	6,500	42	22	54	3	0	1
9/6	8,600	33	42	51	14	0	2
9/7	7,100	21	48	69	8	0	2
9/8	5,800	22	73	71	7	0	0
9/9	6,400	20	59	67	10	0	3
9/10	5,600	19	58	72	7	0	2
9/11	8,300	31	77	51	17	0	1
9/12	9,900	27	70	51	21	0	1
9/13	15,600	41	73	26	32	0	1
9/14	10,600	46	70	32	21	0	1
9/15	7,200	54	75	28	17	0	1
9/16	12,400	75	70	8	17	0	0
9/17	6,900	56	80	29	15	0	0
9/18	Dead						

To show the changes that occur in the leukocytic picture in acute tuberculosis 2 rabbits have been selected for illustration. While the degree of variation from normal may differ considerably in different animals the general trend is always in the same direction. In each of the cases illustrated several leukocytic counts are given before inoculation to indicate what was the "normal" for the animal.

In the case of the rabbit with the avian type of infection (Table I) the ordinary routine leukocytic count is given. It will be noted that

following inoculation the eosinophiles and basophiles gradually drop out of the picture. The balance between the neutrophiles and lymphocytes is not greatly altered for several days, except that it appears as though the neutrophiles tend to recede to or below the lower percentage of normal. Toward the end however the balance is definitely the reverse of normal. From the 10th day on, the monocyte enters the picture definitely. In the tabulation of monocytes we have included in this group all mononuclear cells that evidently did not belong to the other leukocytic types. We believe that among these cells there are many marrow stem cells and young megakaryocytes. Such cells could not, however, be unequivocally differentiated from immature monocytes and they have all been included under the heading of monocytes. An occasional mitotic figure was seen in cells of this type in the blood smears. Thus it is seen that during the evolution of the infection a profound change occurred in the intercellular relation which before death led to a notable increase, proportionally, of neutrophiles and monocytes at the expense of the lymphocytes.

In the case of the rabbit infected with virulent bovine tubercle bacilli (Table II) we have added the immature neutrophiles to the leukocytic count. The percentage in this instance represents that portion of the total neutrophiles which have non-segmented nuclei. This is a modified Schilling count in which stress is placed on the presence in the circulation of immature or non-segmented neutrophiles. A comparison of the interrelation of the leukocytic types before and after inoculation shows the same general trend of events as was seen in the rabbit infected with the avian tubercle bacillus. The difference in the 2 rabbits is only a matter of the degree of change. The addition of the percentage of immature neutrophiles adds an important feature not apparent in the other case. During the period (9-7 to 9-10) when the percentage of neutrophiles has decreased and that of the lymphocytes increased, it is evident that a considerable rise has occurred in the percentage of immature neutrophiles. As the disease progresses and the neutrophile-lymphocyte balance becomes the reverse of normal, the immature neutrophiles continue at a high level. It can readily be seen that in acute tuberculosis in the rabbit it is essential to take into account the immaturity of the neutrophiles, otherwise one would be led to believe that these cells did not enter into the pathological process.

Enumeration of the blood platelets was attempted. It was discontinued because it was felt that with the great variation in the size of the platelets, noticeable as the disease progressed, the number did not give a true indication of the volume of platelet material. From fixed blood smears it was evident that there was at times a considerable increase in the volume of platelets.

Erythrocytic counts were followed in some of the animals, a small proportion of which developed a definite anemia, but this was not a constant feature. A distinct change always occurred in the erythrocytic picture as seen on fixed smears. Nucleated red cells were often numerous. Changes in size, shape and staining reaction of the erythrocytes was always evident toward the end of the infection. Red cells phagocytosed by monocytes were commonly seen in the late stages of the disease.

Since the "systems" of hematopoiesis gave a somewhat different picture in vaccinated reinfected rabbits from that found in the animals with a primary infection, it is of interest to note the change in the circulating blood in the vaccinated reinfected groups. For purpose of illustration an animal vaccinated intravenously with heat-killed bovine type of bacillus and later infected with living organisms of the same strain has been chosen. Animals so treated vary considerably as to the degrees of change in the leukocytic picture, though in general the same trend is noted in all of them. A study of Table III shows the following points of significance: The principal change following the first injection of vaccine was a rise in immature neutrophils which gradually subsided. Following the second inoculation of vaccine the immature neutrophilic picture again changed and in addition there developed a leukocytosis, a definite increase of monocytes, and a drop in percentage of lymphocytes. All of these returned to a normal status before the injection of the living bacilli. Following the injection of the living bacilli the changes noted above not only recurred but persisted. The fluctuation found in the percentages of immature neutrophils during the progress of the disease emphasizes the fact that care must be exercised in the interpretation of this phenomenon in infections of long standing. The picture shifted to what may be termed a septic leukocytic picture within a month after the virulent bacilli were injected and so continued to the death of the animal. Toward the end the picture was such as might be found in infections produced by staphylococci and pneumococci.



TABLE III

*Black Rabbit. Vaccinated Intravenously with Heat-killed Bovine Tubercle Bacilli.  
Later 1 mg. Virulent Bovine Tubercle Bacilli Injected Intravenously*

Leukocytic record							
Date	Total count	Neutrophils	Non-segmented neutrophils 100 cells counted	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%	%
8/22/33	7,700	30	13	60	5	1	4
8/24	11,100	34	15	52	4	3	7
8/26	9,100	38	48	42	5	6	9
8/28	10,600	40	27	48	5	4	3
(8/28	5 mg. heat-killed bacilli intravenously)						
8/29	10,600	38	45	44	10	3	5
8/30	16,100	28	46	60	7	1	4
8/31	6,900	33	28	53	10	1	3
9/2	6,000	34	30	55	6	1	4
9/4	7,400	44	23	47	7	1	1
9/6	8,400	38	34	42	14	1	5
9/9	11,700	47	22	29	11	9	4
9/13	13,900	17	18	62	12	0	9
(9/13	10 mg. heat-killed bacilli intravenously)						
9/14	30,500	60	53	25	8	0	7
9/15	15,400	31	29	39	24	1	5
9/16	18,500	39	35	35	22	0	4
9/17	20,000	40	52	34	20	0	6
9/19	17,000	39	28	42	15	0	4
9/21	21,400	37	19	41	16	3	3
9/24	8,500	37	24	56	6	0	1
9/27	6,300	48	26	42	7	1	2
9/30	7,700	25	28	64	8	0	3
10/4	14,500	25	24	50	23	1	1
10/8	4,500	23	18	67	7	0	3
10/12	7,300	31	26	53	12	2	2
10/15	12,300	37	22	48	11	0.5	3.5
10/19	7,800	31	27	55	7	2	5
10/23	10,600	31	26	62	5	1	1
10/27	7,000	30	14	56	10	0	4
11/1	7,000	18	22	75	5	0.5	1.5
11/3	7,700	34	18	57	6	1	2
11/6	10,500	48	16	34	12	1	5
11/8	19,000	38	18	33	18	0	11
11/10	9,800	35	11	55	5	0	5
11/13	9,000	46	17	46	5	0	3
(11/13	1 mg. living virulent bovine tubercle bacilli intravenously)						
11/14	11,800	47	54	40	6	0	7
11/15	17,300	17	25	65	15	0.5	2.5
11/17	6,600	35	58	48	12	1	4
11/20	17,200	40	45	40	17	0	3
11/22	8,300	23	60	58	12	5	2
11/24	34,000	39	62	36	20	0	5
11/27	9,200	29	62	50	19	0	2

TABLE III (Continued)

Leukocytic record							
Date	Total count	Neutrophils	Non-segmented neutrophils 100 cells counted	Lymphocytes	Monocytes	Eosinophiles	Basophiles
		%	%	%	%	%	%
11/29/33	6,600	36	70	38	25	0	1
12/1	16,000	27	67	41	31	0	1
12/4	63,300	56	67	17	24	0	3
12/6	23,200	39	67	26	30	0	5
12/8	17,600	74	54	17	7	1	1
12/11	9,200	52	54	35	10	0	3
12/13	9,900	55	51	32	11	1	1
12/15	7,500	48	54	40	10	0	2
12/18	51,000	73	60	12	13	0	2
12/20	7,000	63	60	29	7	0	1
12/22	23,100	58	47	23	18	0	1
12/26	8,300	55	45	35	9	0	1
12/28	12,100	67	61	21	11	0.5	0.5
12/30	18,100	60	37	25	13	0	2
1/2/34	11,300	47	40	26	23	0	4
1/4	9,800	70	31	16	14	0	0
1/8	16,100	56	44	21	20	1	2
1/10	9,500	57	38	25	16	1	1
1/12	6,000	61	32	26	11	2	0
1/15	12,300	50	42	19	29	0	2
1/17	17,700	69	28	14	16	0.5	0.5
1/19	30,000	78	45	7	15	0	0
1/22	12,400	76	34	11	11	0	2
1/24	9,600	69	45	15	15	0	1
1/26	23,400	69	33	13	17	0.5	0.5
1/29	11,600	72	20	13	14	0.5	0.5
1/31	10,900	73	32	16	11	0	0
2/3	34,000	70	26	14	15	0	1
2/5	18,400	71	42	13	15	0	1
2/7	23,000	65	48	10	24	0	1
2/9	18,000	72	55	13	14	0.5	0.5
2/12	23,800	69	40	13	18	0	0
2/15	34,000	68	50	12	20	0	0
2/19	27,100	85	30	3	10	0	2
2/22	16,800	81	50	10	7	0	2
2/24	Dead						

The shifting about of the leukocytic picture indicates not only the delicate interplay of cellular function but it also emphasizes the fact that a single leukocytic count can reveal only the status of the pathological process at the time the count is taken. Thus leukocytic counts taken at different times may reflect the different phases of a pathological process within the body.

Thus it will be seen that the circulating blood gives definite evidence of a profound reaction in the hematopoietic tissues as the disease progresses. In all of the animals the shift in the circulating blood picture lagged behind the changes found in the quasi-fixed portions of the system.

To complete the brief comparison we have been making between staphylococcal and tuberculous infection the leukocytic record of 2 rabbits given an intravenous injection of *Staphylococcus aureus* are appended. The 2 rabbits selected for illustration were given the same dosage of cocci on the same date. One (Table IV) died within 5, and the other (Table V) within 17 days.

TABLE IV

*Rabbit No. 1. 1 mg. Staphylococci Injected Intravenously*

Leukocytic record							
Date	Total count	Neutrophils	Immature neutrophils 100 cells counted	Lymphocytes	Mono-nuclears	Eosinophiles	Basophiles
		%	%	%	%	%	%
1/24/33	3,900	52	15	34	8	2	4
1/25	8,000	59	16	28	7	2	4
1/26	6,500	60	20	26	8	2	4
1/27	8,600	28	18	59	9	0.5	3.5
1/29	5,500	58	13	33	5	1	3
1/30	2,500	50	20	33	7	1	9
(1/31 Staphylococci injected)							
1/31	5,100	40	19	41	9	1	9
2/1	8,900	89	41	5	3	1	2
2/2	17,200	68	33	4	12	0	16
2/3	16,600	81	44	5	8	1	5
2/4	2,600	73	63	8	10	1	8
2/5	Dead						

Tables I to V record the variations observed in the circulating leukocytic picture in different types of infection. The differences observed in animals given the same type of infection are given. Also the effect of changes in the tissues through vaccination on the leukocytic response is depicted. A careful study of the leukocytic records reveals a considerable variation in the interplay between the various leukocytic types both prior to and after the introduction of the infectious agent. The degree of change is much greater after the injection of the bacteria.

*Correlation of the Cellular Interplay*

The changes that have occurred in the hematopoietic tissue have been presented under the headings of "systems" so that it would be possible to describe the successive alterations that occurred in each "system." To correlate the findings in the hematopoietic tissue as a whole we now take a single animal (Table II) and show what has

TABLE V

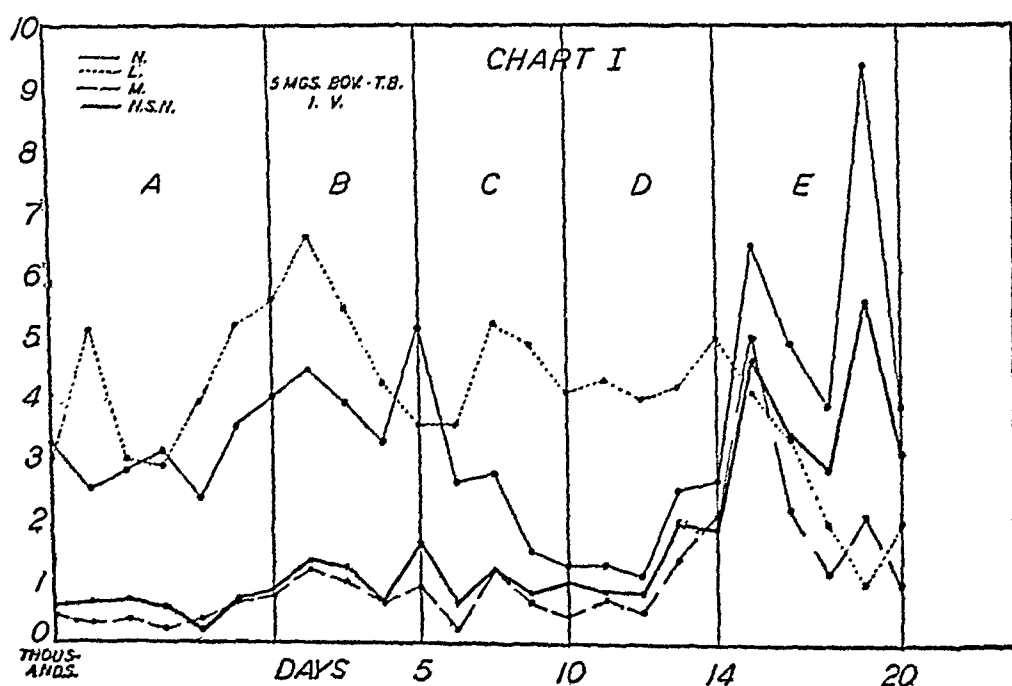
*Rabbit No. 3. 1 mg. Staphylococci Injected Intravenously*

Leukocytic record							
Date	Total count	Neutrophiles	Non-segmented neutrophiles 100 cells counted	Lymphocytes	Mono-nuclears	Eosino- philes	Baso- philes
		%	%	%	%	%	%
1/24/33	9,700	42	23	47	9	1	1
1/25	9,700	37	21	56	5	1	1
1/26	11,700	36	27	53	9	..	2
1/27	12,700	42	23	52	5	..	1
1/29	9,700	32	12	60	6	..	2
1/30	12,200	33	12	61	4	1	1
(1/31 Staphylococci inoculated intravenously)							
1/31	9,700	43	16	52	3	1	1
2/1	12,500	74	51	16	5	..	5
2/2	26,100	45	44	15	30	..	10
2/3	25,400	53	49	21	19	0.5	6.5
2/4	31,700	50	54	16	18	..	16
2/5	20,400	42	57	11	21	..	26
2/6	43,700	39	56	16	29	..	16
2/7	33,000	62	55	8	24	..	6
2/8	39,600	59	52	14	23	..	4
2/9	21,200	63	52	11	21	..	5
2/10	41,000	67	57	12	15	..	6
2/11	36,000	76	52	8	15	..	1
2/12	113,000	86	55	3	11	..	..
2/13	39,300	76	40	7	15	..	2
2/14	43,000	81	59	4	12	..	3
2/15	100,800	89	73	4	7	..	..
2/16	20,600	84	76	6	9	..	1
2/17	Dead						

been the hematopoietic response during the course of the disease. In Chart 1 graphs of the total number of the different leukocytic types known to participate in the tuberculous lesions are given. It will be noted that with the progress of the infection a marked alteration occurs in the interrelation of the different cell types. We have divided (A, B, C, D and E) the story told by the circulating leuko-

cytes to correspond to the periods of observation of the quasi-fixed portion of the hematopoietic tissue.

In Table VI the picture of the hematopoietic tissue as a whole is given. In the first part of the table the proportional relations of the different leukocytic types shown in Chart 1 are given, using the monocyte as the basal unit. Here we have used the average for all observations made during each division (A, B, C, D and E) in the chart. In the second portion of the table a composite picture of the condition of each "system" is given for each division of the chart. From the table it is evident that it takes several days for the blood to



"mirror" the changes occurring in the quasi-fixed portion of the blood-forming tissues. Thus the changes in the circulating blood in C reflect the changes in the spleen, marrow and lymphoid tissue in B, and so on. It has been our experience that this lag continues and that evidence of lessened hyperplasia occurs in the hematopoietic tissues before such a phenomenon is observed in the circulating blood.

Chart 1 and Table VI clearly show that the hematopoietic tissue as a whole is involved in the response to the tuberculous infection. As the disease progresses, shifts in the degree of response of the different "systems" occur and, allowing for a lag, these shifts become evident in the circulating blood. Thus the hematopoietic response to tuber-

TABLE VI

*A Correlation of the Changes Occurring in the Different Portions of the Hematopoietic Tissue During the Progress of Tuberculous Infection*

Division of Chart I	Proportional relations of circulating leukocytes				Bone marrow	Spleen	Lymphoid tissue of appendix	Reticulo-endothelium of liver
	Neutrophiles	Non-segmented neutrophiles	Lymphocytes	Monocytes				
A	6.79	1.20	9.11	1.00	Normal Fig. 1. Early hyperplasia.	Normal Figs. 16 & 18 Early hyperplasia.	Normal Figs. 29 & 32 Early hyperplasia.	Normal
B	4.51	1.27	5.32	1.00	Fig. 2. Scattered cells containing tubercle bacilli	Figs. 20 & 21. Rare tubercle	No tubercles	Normal. Early tubercle formation. Fig. 13
C	3.46	1.46	7.37	1.00	Marked hyperplasia. Fig. 3. Rare tubercle	Marked hyperplasia. Figs. 22 & 23. A few tubercles	Marked hyperplasia. No tubercles	Normal. Numerous tubercles. Fig. 14
D	1.67	1.17	3.80	1.00	Marked hyperplasia. Figs. 4, 5 & 6. A few tubercles	Marked hyperplasia. Figs. 24 & 25. Tubercles numerous	Marked hyperplasia. Figs. 30, 33 & 34. No tubercles	Normal. Tuberculosis more extensive
E	2.47	1.71	1.08	1.00	Marked hyperplasia. Figs. 7, 8 & 9. Extensive tuberculous	Hyperplasia not so marked. Figs. 17, 26, 27 & 28. Tuberculosis extensive	Hyperplasia less evident. A rare tubercle	Normal. Extensive tuberculous. Fig. 15

culous infection is not with one cell type but rather with an interplay of several cell types.

### COMMENT

It is the purpose of this paper to demonstrate that the introduction of virulent tubercle bacilli into the animal body creates a complex condition that demands an interplay of various cell types of the hematopoietic tissue to counteract the damage produced. That is, the pathology of tuberculosis is not a one cell type of pathology. It is evident that the cells that constitute tuberculous lesions come in large part from the hematopoietic tissue. The changes that occur in the quasi-fixed areas of hematopoiesis are then of fundamental importance.

Since different cell types of the blood are involved it is necessary to consider the hematopoietic tissue as a whole. Due regard must be given to the widespread distribution of the specialized "systems" that comprise the tissue, to the inherent complexity of each "system" because of the pluripotentiality of the primitive or "stem" cells, to the essential mobility of the tissue, and to the various cell types that are components of the mobilized portion (the circulating blood) of the tissue.

The data in the text demonstrate clearly that the marrow, the spleen and the lymphoid tissue respond early and intensely to the infection. The "reticulo-endothelial system," if one may judge from the reaction in the liver, plays little if any part. The fundamental response in the "systems" reverts early to the primitive blood cells. This renders an interpretation of the hematopoietic response difficult because at present there are no technical methods that make possible the positive identification, or certain the maturation process, of any one primitive cell. A great deal of the confusion that now exists in the terminology of hematology rests here. Too great zeal to "tag" primitive blood cells should be discouraged until such time as positive identification can replace theoretical concepts.

As time elapsed it became evident that the principal demands were for neutrophils and megakaryocytes from the bone marrow, and for monocytes from the spleen and lymphoid tissue. There was also evidence in some animals that increased erythrocytic production from the bone marrow was required. For some time the production of lymphocytes did not seem to be affected. Later in the infection

the evidence pointed to a continued demand for neutrophils with a lessening of the need for monocytes, lymphocytes and megakaryocytes. This same sequence of events was found to occur in animals that had been partially immunized by vaccination, with the exception that the hematopoietic response was more rapid than in non-vaccinated rabbits.

There is a general belief that the myelogenous portion of the hematopoietic tissue does not play a prominent rôle in the body's reaction to pure tuberculous infection. Whenever this tissue has been found to participate, the reaction has been attributed to the presence of secondary invaders, such as streptococci, pneumococci, and so on. Cunningham *et al.*,<sup>2</sup> have noted that in experimental animals the bone marrow is often found to be hyperplastic in the later stages of tuberculosis. They have attributed this condition to an over-regeneration following a depression of marrow function caused by the presence of tuberculous infection in the tissue. Our observations are that marrow hyperplasia begins shortly after the inoculation of tubercle bacilli and before definite tubercles are present. This hyperplasia continues until the death of the animal even if the marrow becomes the site of extensive tubercle formation. We have not obtained any evidence suggestive of a depression of hematopoietic activity in this tissue.

The intense response found in the spleen and lymphoid tissue of the vermiform appendix suggest that in acute tuberculous infection these tissues are concerned primarily with the production of monocytes. It appears that the primitive cells of this portion of the hematopoietic apparatus differentiate into monocytes. Whether or not the primitive cells of lymph nodes can also mature in this same direction we cannot state with certainty. We found no evidence that the monocyte of the rabbit is produced in the marrow and liver or from "fixed tissue cells." Our findings correspond closely to those of Bloom.<sup>3</sup> We do not believe, as Bloom does, that the differentiated lymphocytes can become transformed into monocytes but we do believe that the primitive cell may be the same for the lymphocyte and the monocyte. Witts and Webb<sup>4</sup> regard the monocyte as originating from the marrow tissue as well as from the spleen. They state that the monocytes of the marrow are "less mature than in the spleen." We wonder if the monocyte can be correctly identified in such an immature state as the one they observed in marrow preparations



and if they may not have been dealing with the very early stages of other marrow cell types, perhaps the megakaryocyte. Naegeli,<sup>5</sup> and others, maintain that the monocyte originates in the myelogenous tissue. Until it becomes possible to identify unequivocally the stem cell, from which the monocyte develops, differences of opinion will remain as to whether or not this cell type really does have a myelogenous origin. Our data are against such an origin in the rabbit infected with the tubercle bacillus.

Evans, Bowman and Winternitz,<sup>6</sup> Aschoff,<sup>1</sup> Cunningham *et al.*,<sup>2</sup> Foot,<sup>7</sup> Permar,<sup>8</sup> and others, regard the monocyte as arising from the "reticulo-endothelial system." From our studies we have become skeptical as to the existence of such a "system." The essential mobility of the blood tissue and the fact that immature and even primitive blood cells, which retain their propagating propensities, enter the circulating blood, render the presence of mitotic figures and the location of blood cells in sections of tissue of doubtful significance relative to the origin of such cells.

The changes we have found in the circulating blood picture correspond closely to those reported by Cunningham *et al.*,<sup>2</sup> and others. One point which the various reports have not stressed, and which we believe to be significant, is the definite increase in the proportion of immature neutrophils as the tuberculosis progresses. This phenomenon we have found to be extremely pronounced, even when a severe neutropenia existed. Failure to recognize the occurrence of this great "shift" in the neutrophilic picture has probably been largely responsible for the dictum that neutrophils do not play a significant rôle in pure tuberculous infection. In previous communications<sup>9, 10, 11</sup> attention has been called to the important part played by the neutrophile in certain phases of the pathogenesis of tuberculosis. The presence of the definite "shift to the left" of the neutrophilic picture in the circulating blood further emphasizes the observations we have previously reported.

From our blood studies on tuberculous animals that have lived for several months we have found that there is a distinct tendency for the proportion of immature neutrophils to decrease after the 1st month, even though the animals eventually died of the infection. On occasion we have observed that the proportion of non-segmented neutrophils had returned to normal, although the total number of neutrophils was well above normal. We believe this to be due

to the fact that the myelogenous tissue had expanded sufficiently so that neutrophiles could be matured in large enough numbers to meet the demand. This would indicate that a decrease in the immature neutrophiles in the circulating blood does not necessarily signify an improvement in the pathological process. This emphasizes the fact that the leukocytic picture as a whole, rather than any one portion of it, should always be considered.

We have found it difficult to make accurate differential leukocytic counts as the acute tuberculous infection progressed, since cells so immature as to render identification mere guesswork entered the circulation. In the tabulations we have included these immature cells with the monocytes. We believe that the large majority of such cells were either undeveloped megakaryocytes or monocytes. Such cells make up from 5 per cent to 20 per cent of the cells classed as monocytes.

As mentioned in the text, changes in the circulating blood lag behind those in the quasi-fixed hematopoietic tissues. This is especially true after the available supply of matured cells is used up during the first 3 or 4 days following the introduction of the tubercle bacilli. Although a lag occurs, the circulating blood in time "mirrors" the changes that have occurred in the bone marrow, spleen and lymphoid tissue.

During the evolution of a pathological process conditions are not static. At one stage the picture found may bear little resemblance to that of another period. This is especially true in circumstances where the hematopoietic tissue is involved, as is shown by the data presented in the text. Not only does the appearance of the different portions of the hematopoietic system vary from time to time in a single type of infection, but it differs even more in different kinds of infection. There is, as well, a considerable variation among individuals with the same type of disease which must be taken into account. Since these conditions exist, the picture found on the examination of a blood smear or of a section of marrow or lymph node should be interpreted as representative of a certain phase of a pathological process rather than as diagnostic of the whole process.

The cellular complexity, together with numerous maturation processes, makes it practically impossible to give a simple and coordinated description of the changes that have occurred in the whole hematopoietic tissue during the evolution of lethal tuberculosis.

We have purposely avoided the coining of new names and have attempted to remain within the realm of clearly demonstrated facts. The creation of elaborate classification, the coining of new names to label cells at frequent intervals during the maturation process and at different stages of functional activity, and the dividing of the hematopoietic tissue into many "systems" we believe simply adds confusion to the understanding of a tissue that nature has left in a topsy-turvy state — perhaps for a reason.

Regardless of the fundamental complexity of hematopoiesis, the important part played by the blood-forming tissue in many and diverse pathological conditions requires that greater effort be made to coördinate the changes that occur. The data we have presented in the text show beyond question that in serious tuberculous infection all parts of the hematopoietic apparatus are affected in one way or another. Also we have shown that a very definite interplay of the different cell types occurs during the progression of the infection. We believe that in all studies where the hematopoietic tissues are under consideration this tissue should be investigated as a whole rather than to examine one or another "system" and ignore the rest. Such investigations should always include careful studies of the circulating blood. One fact that remains paramount is that the blood is in essence a mobile tissue and that the "fixed tissue" portion is only quasi-fixed — factories from which the circulating blood is replenished.

#### SUMMARY AND CONCLUSIONS

From our studies of the complex changes and of the interplay of different cell types in the hematopoietic tissue during the progression of acute tuberculous infection in rabbits we can briefly summarize the conditions found as follows:

1. All systems of the hematopoietic tissue are involved.
2. The stem cells are greatly increased.
3. Undifferentiated cells, perhaps stem cells, enter the circulation. These cells retain the ability further to multiply within the circulating blood and also in all probability after entering tissues other than blood-forming. This adds greatly to the uncertainty as to whether a cell in mitosis has arisen *in situ* or in some far removed portion of the hematopoietic tissue.
4. The cells in the germinal centers of the spleen are probably

stem cells which, in acute tuberculosis in the rabbit, differentiate into monocytes. It also seems probable that the pulp cells of the spleen have the same origin.

5. It seems probable that monocytes also originate from at least some portions of the lymphoid tissue outside the spleen.

6. No evidence was found to suggest that the monocyte arises from the marrow or from the reticulo-endothelial system outside of the lymphoid system.

7. The criteria on which the concept of a reticulo-endothelial system rests are insufficient to establish such a system.

8. The circulating blood mirrors the changes found in the so-called fixed hematopoietic tissues, although there may be a lag of several days before changes are reflected.

9. The interplay of various cell types of the hematopoietic tissue necessitates a consideration of this tissue as a whole in pathological processes where it may play an important rôle. Single observations should be regarded as indicative of a phase in a pathological process rather than as characteristic of the process as a whole.

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### DESCRIPTION OF PLATES

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In all of these experiments tubercle bacilli were inoculated intravenously.

#### PLATE 133

- FIG. 1. Central portion of femur marrow of a normal rabbit. This portion of the marrow is normally composed in large part of fat cells. Hematopoietic tissue in this area is made up largely of erythrogenic tissue with a scattering of other cell types.  $\times 500$ .
- FIG. 2. Central portion of femur marrow 5 days after inoculation of avian tubercle bacilli. There is an increase of hematopoietic tissue. Myelocytes, megakaryocytes and stem cells definitely increased. No tubercles found. Occasional mitotic figures are present.  $\times 300$ .
- FIG. 3. Central portion of femur marrow 10 days after injection of avian tubercle bacilli. Tissue intensely hyperplastic. Mitoses abundant—three present in illustration. Cells predominantly of the stem cell variety. Rare small monocytic tubercles are present at this stage.  $\times 800$ .



PLATE 134

FIG. 4. Femur marrow 14 days after injection of avian tubercle bacilli. Extensive hyperplasia throughout marrow. There is now no essential difference between cellular content of the peripheral and central portions of the marrow. Fat cells widely separated (appear as clear circles in illustration). Note scattered, well defined tubercles which are largely in the peripheral portion of the marrow.  $\times 100$ .

FIG. 5. High power from Fig. 4. An area in central portion of marrow giving a picture quite similar to Fig. 3. Note megakaryocyte emigrating into blood capillary.  $\times 800$ .

FIG. 6. High power of a tubercle in Fig. 4. Note the contrast in staining reaction of the tuberculous monocytes and the stem cells in Fig. 5. There is a mitosis in a tuberculous monocyte in the upper right hand quadrant.  $\times 800$ .

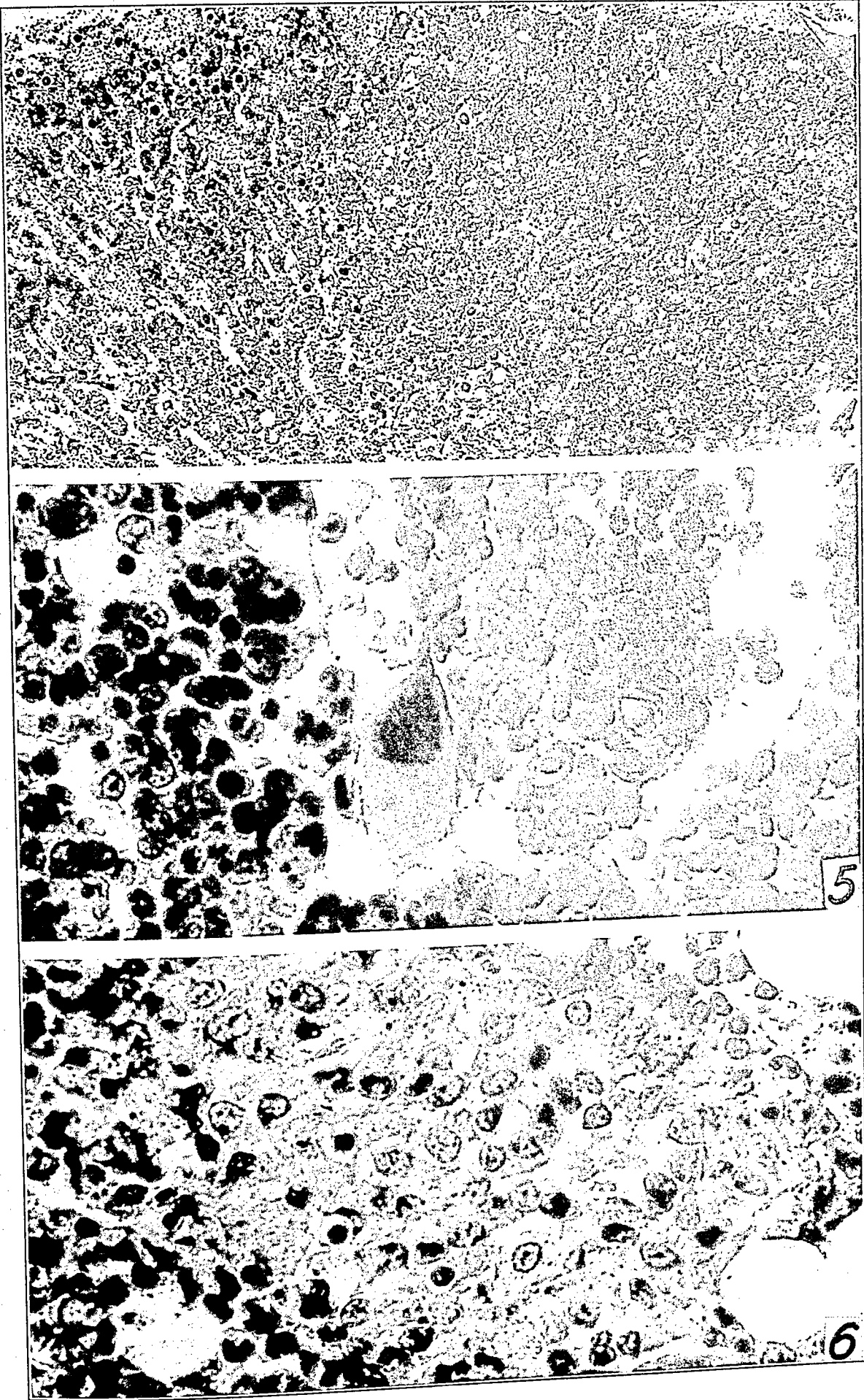




PLATE 135

FIG. 7. Femur marrow 3 weeks after inoculation of avian tubercle bacillus. Note uninvolved hyperplastic marrow at the periphery (upper part of illustration) and the extensive sheets of tuberculous tissue.  $\times 100$ .

FIG. 8. From upper portion of Fig. 7. Note the hyperplastic marrow tissue composed largely of stem cells in the upper portion and the sheet of well preserved tuberculous monocytes in the lower portion.  $\times 500$ .

FIG. 9. From lower portion of Fig. 7. Note the necrosis of the monocytes and a mild infiltration of neutrophils (the irregular deep'y staining masses are the nuclei of neutrophils).  $\times 800$ .

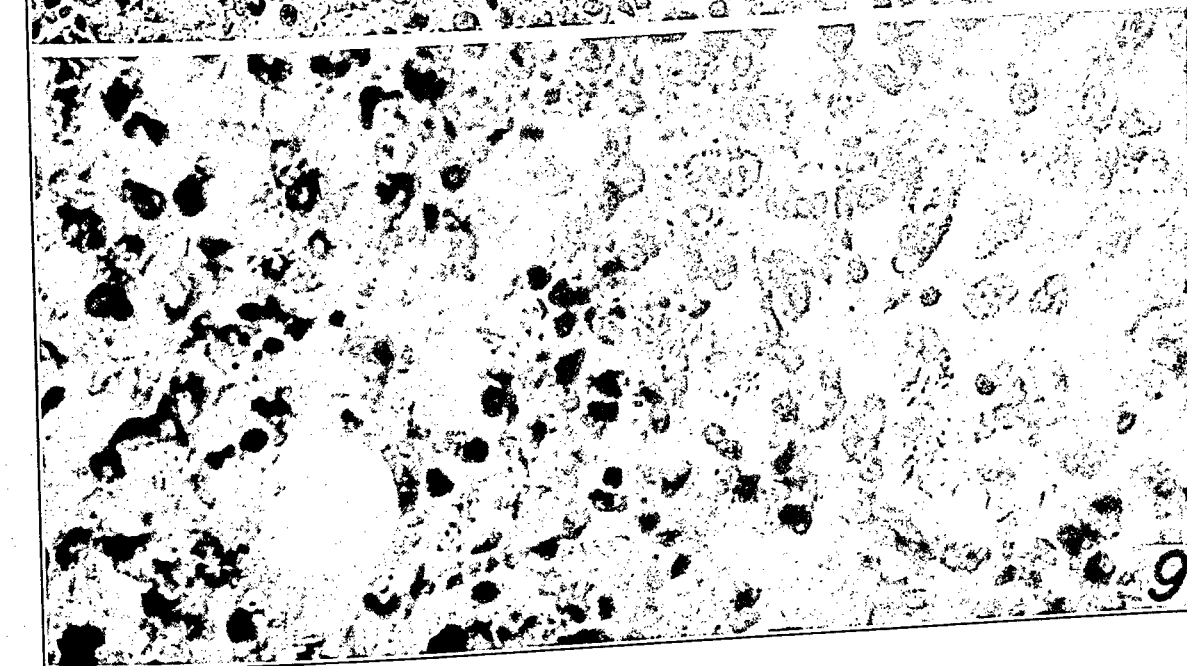
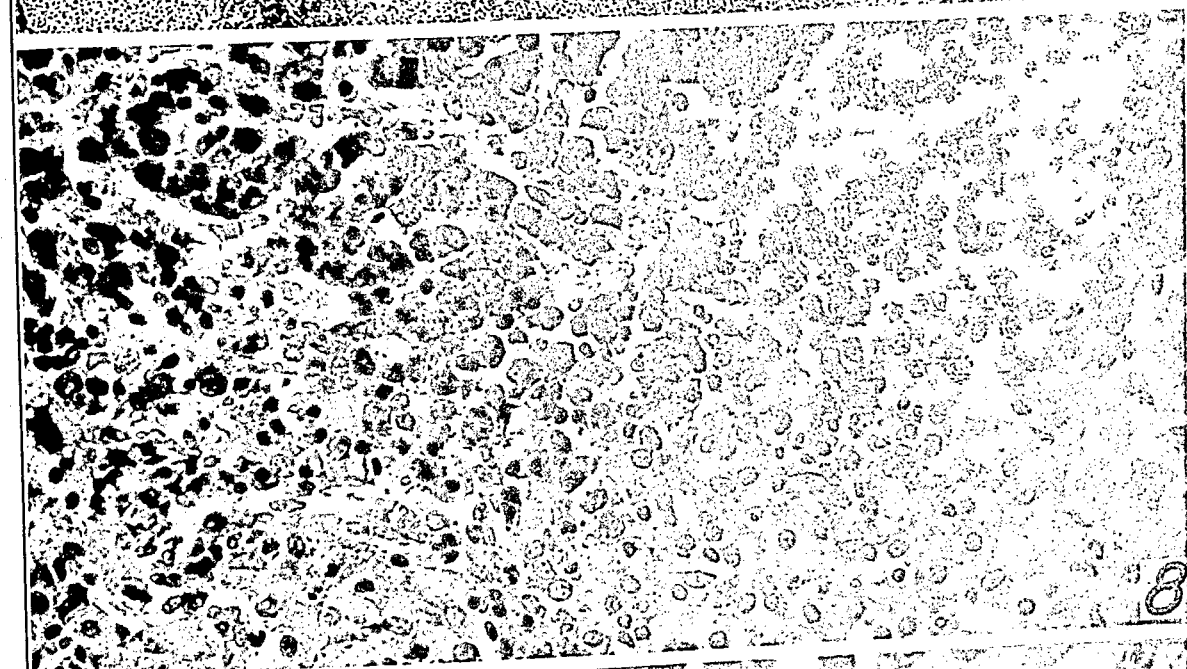


PLATE 136

- FIG. 10. Marrow from a rabbit vaccinated with heat-killed avian tubercle bacilli and later infected with living organisms of the same strain. Animal died 4 months after the injection of the living bacilli. Marrow is devoid of fat and congested. Note the tuberculous lesion adjacent to the large vessel. This lesion is composed of a mixture of monocytes and lymphocytes with a heavy infiltration of lymphocytes about the periphery.  $\times 100$ .
- FIG. 11. An area from Fig. 10. While the marrow is not crowded with cells the hematopoietic tissue present is hyperplastic. There are six mitotic figures in this field, two of which are in focus. Cells are largely of the myelocytic and neutrophilic types.  $\times 500$ .
- FIG. 12. Marrow from an animal infected with 0.01 mg. of highly virulent bovine type of tubercle bacilli. Animal died within 3 months. Note the large tuberculous lesions, the upper one having a caseous center. The uninvolved marrow is similar to that in Fig. 10.  $\times 100$ .

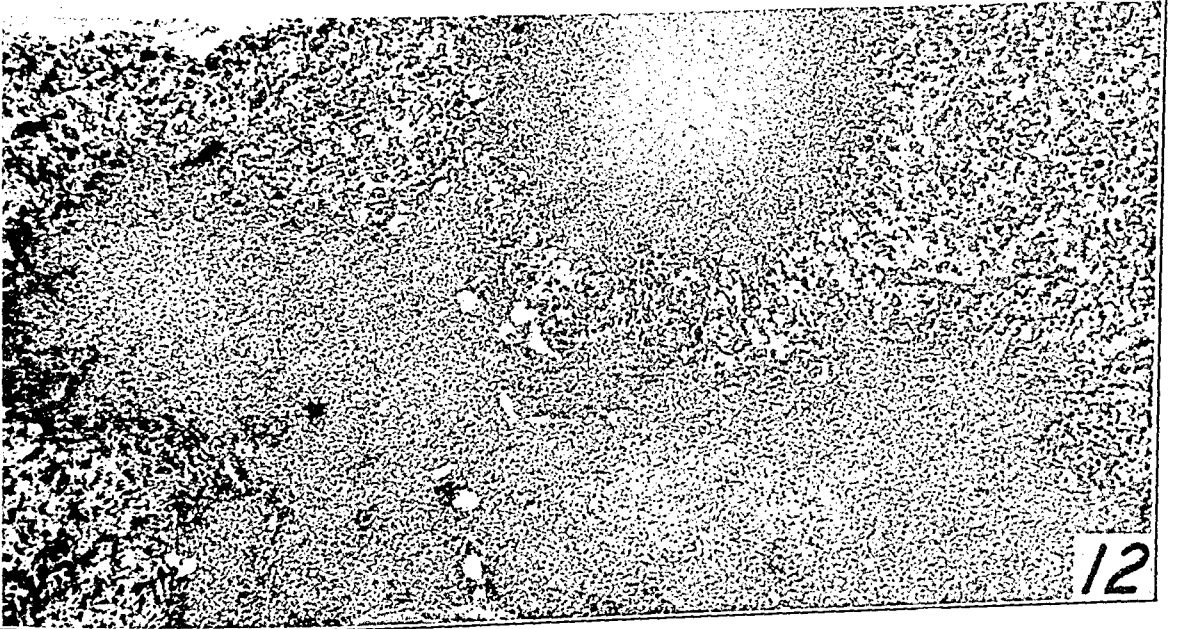
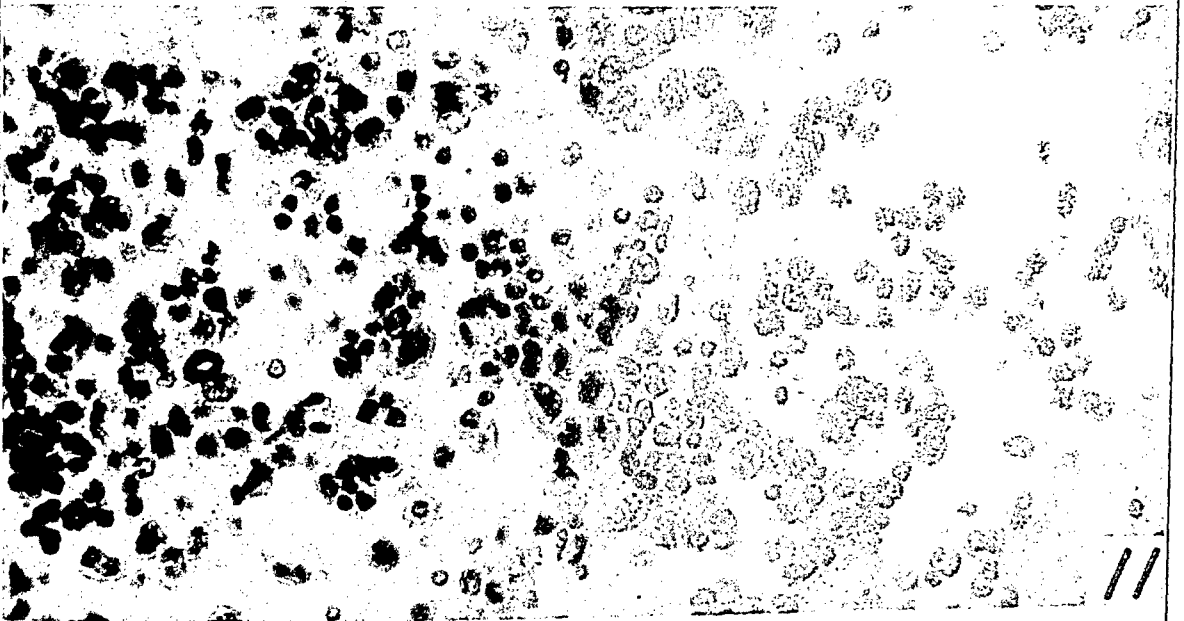
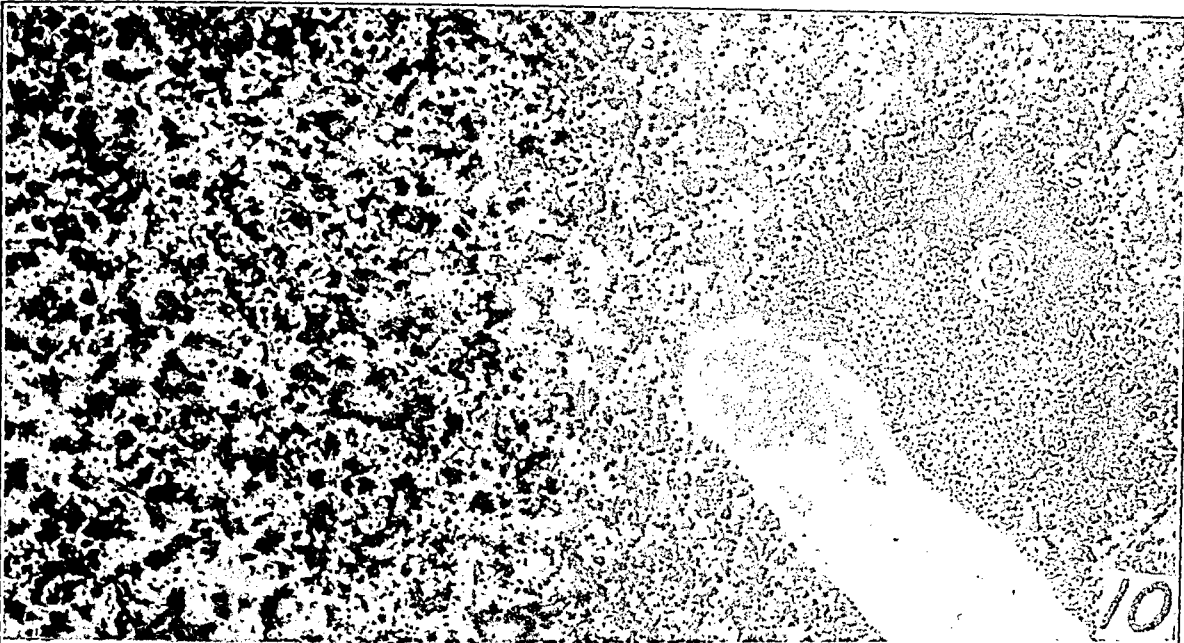


PLATE 137

FIG. 13. Liver 5 days after inoculation of avian tubercle bacilli. Tuberculous lesion is composed of a small intracapillary accumulation of mononuclear cells (upper center). Note that there is no evidence of increase of Küpffer cells outside of tuberculous lesion.  $\times 800$ .

FIG. 14. Liver 10 days after inoculation of avian tubercle bacilli. Note typical monocytic tubercle in central portion. This lesion is larger than in Fig. 13 but the tissue is otherwise similar to it.  $\times 800$ .

FIG. 15. Liver 3 weeks after inoculation of avian tubercle bacilli. Note large intracapillary lesion which shows some necrosis of monocytes and beginning infiltration of neutrophiles. Note that there is no evidence of increase of Küpffer cells in capillaries adjacent to tuberculous focus.  $\times 800$ .

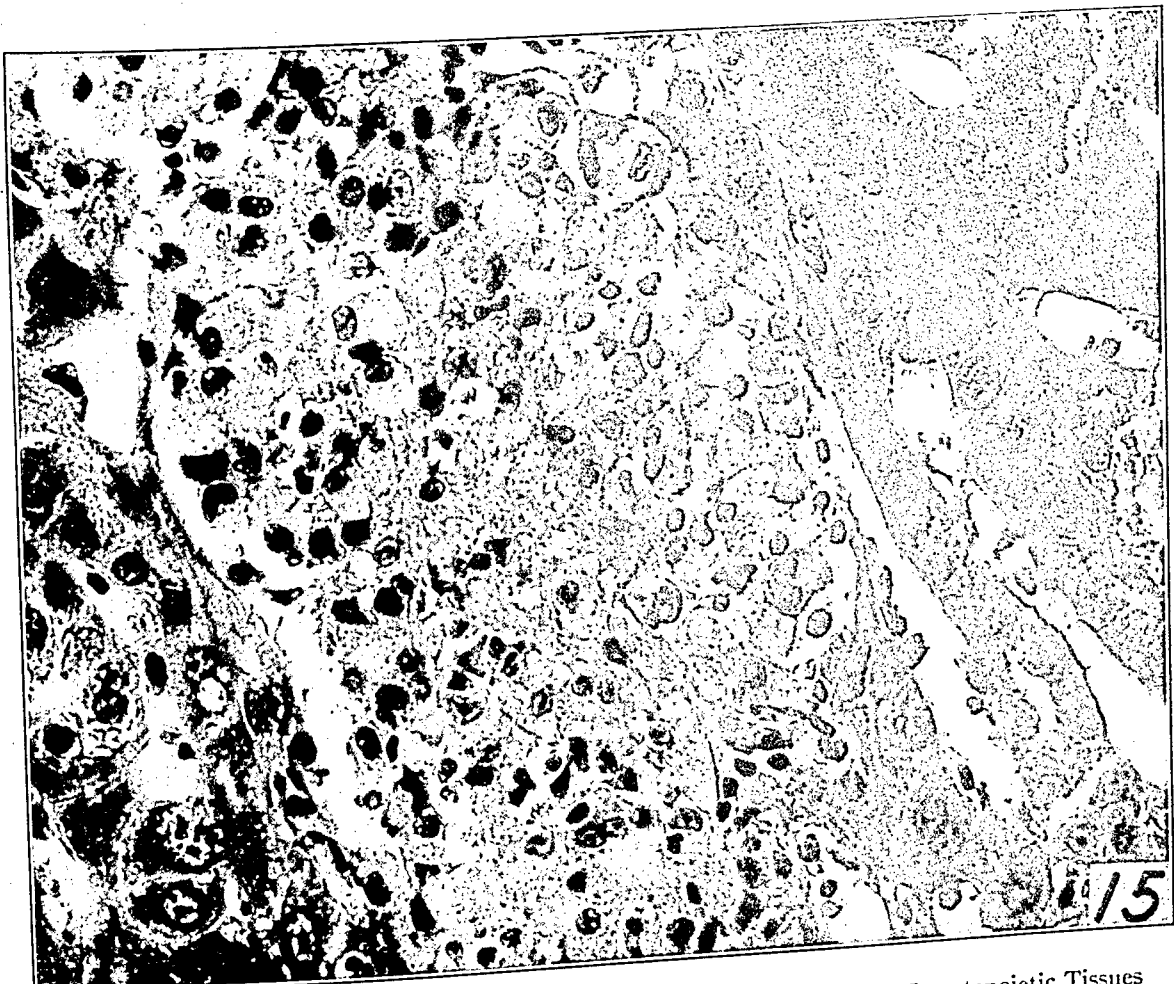
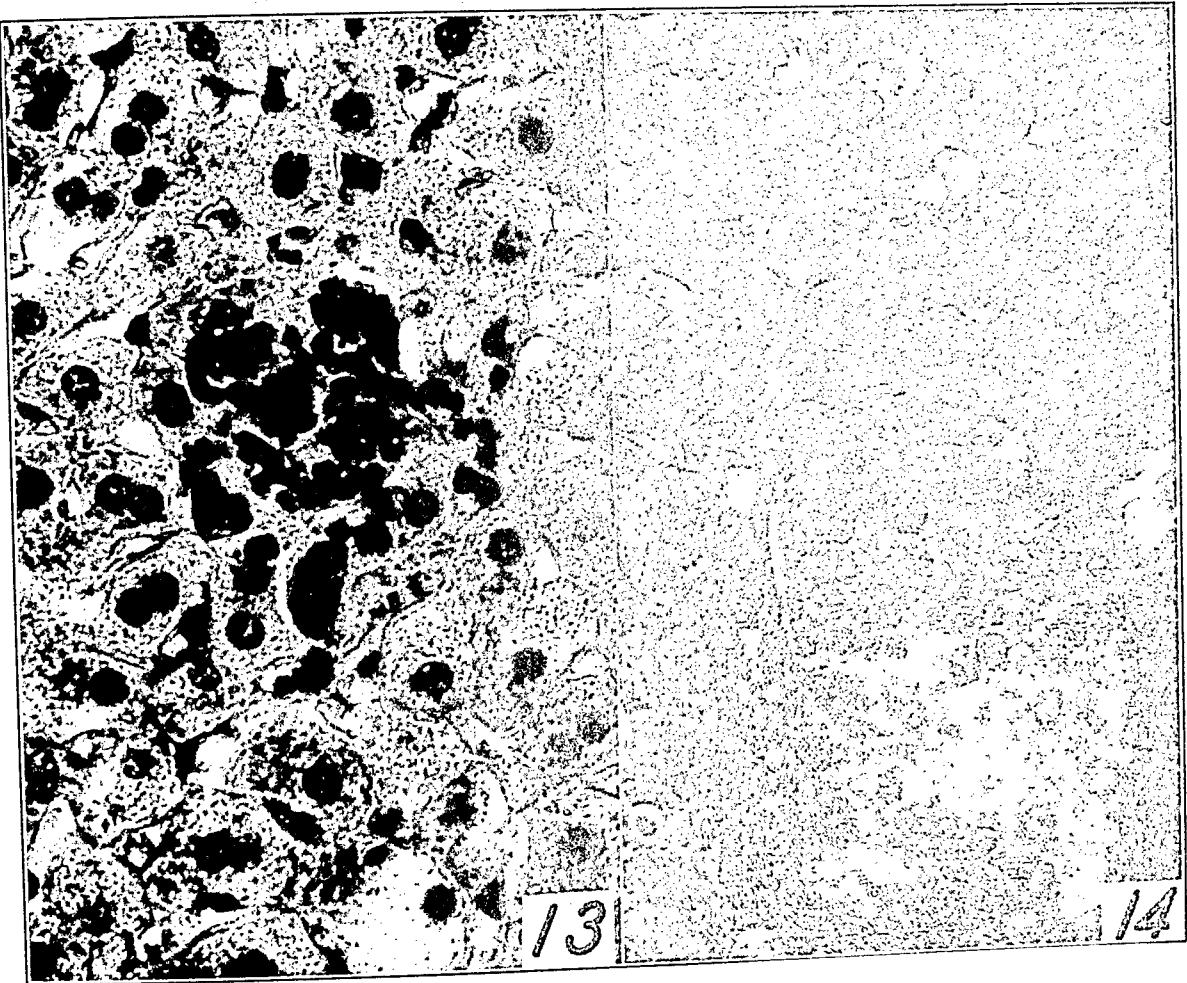


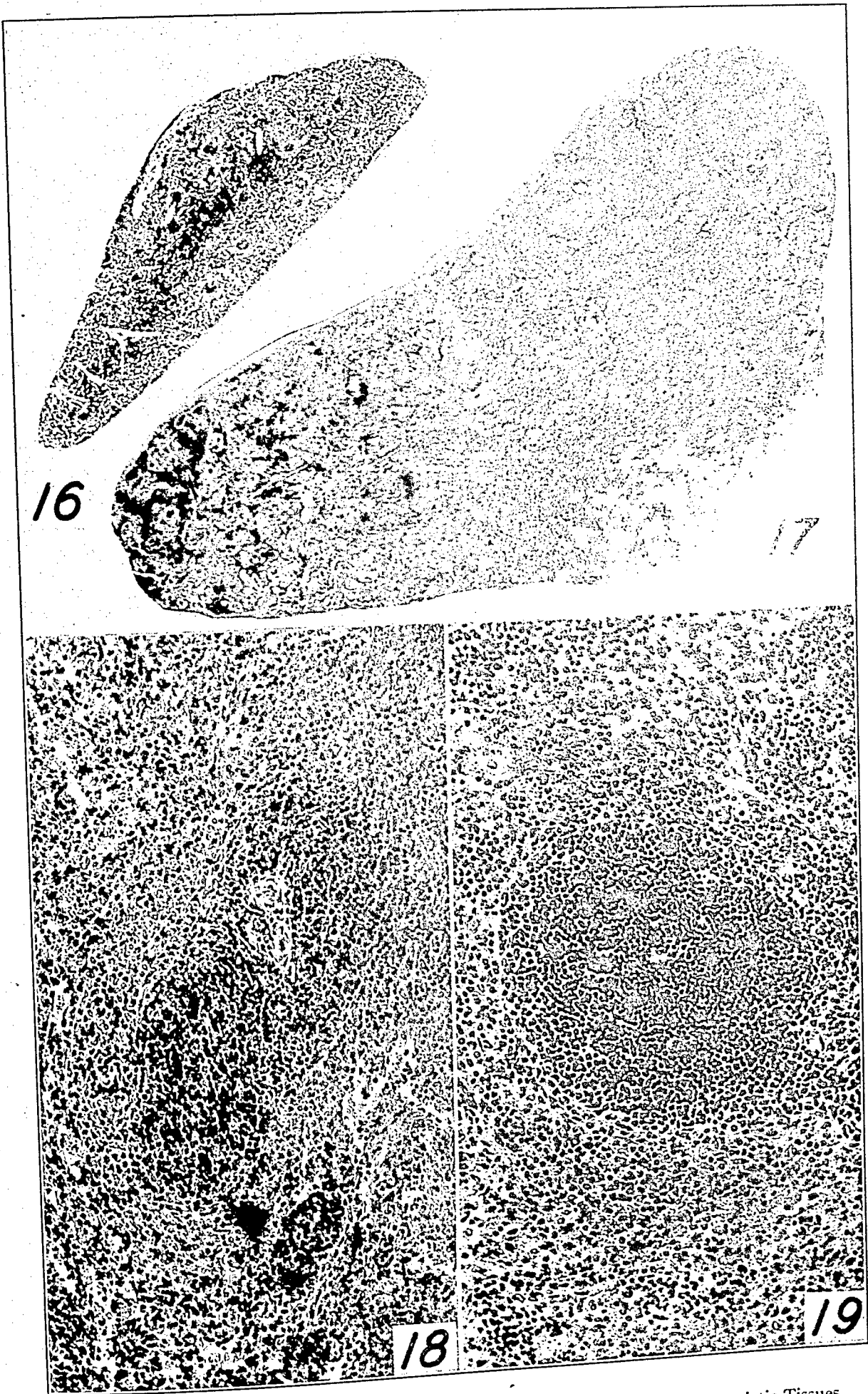
PLATE 138

FIG. 16. Normal rabbit spleen.  $\times 10$ .

FIG. 17. Spleen 3 weeks after inoculation of avian tubercle bacilli. Organ is composed in large part of monocytic reaction to tubercle bacilli.  $\times 10$ .

FIG. 18. Germinal center from Fig. 16. Note the closely packed, small, deeply staining cells in the central portion and the peripheral "collar" of larger, lighter staining cells. Note similarity of cells in pulp and those in the "collar."  $\times 200$ .

FIG. 19. Germinal center in spleen of rabbit that died 5 days after the inoculation of *Staphylococcus aureus*. No evidence of hyperplasia. Compare with Figs. 18, 20, 22 and 24.  $\times 200$ .



Interplay of Cells of Hematopoietic Tissues

Medlar and Sasano



PLATE 139

FIG. 20. Germinal center of spleen 5 days after inoculation of bovine type of tubercle bacilli. Note that the germinal center is enlarged, the "collar" is prominent and the pulp is no more cellular than normal. Compare with Figs. 18 and 29.  $\times 200$ .

FIG. 21. Higher power of Fig. 20. Note two types of cells — small deeply staining, and larger paler staining. Mitotic figures numerous. No tubercles.  $\times 400$ .

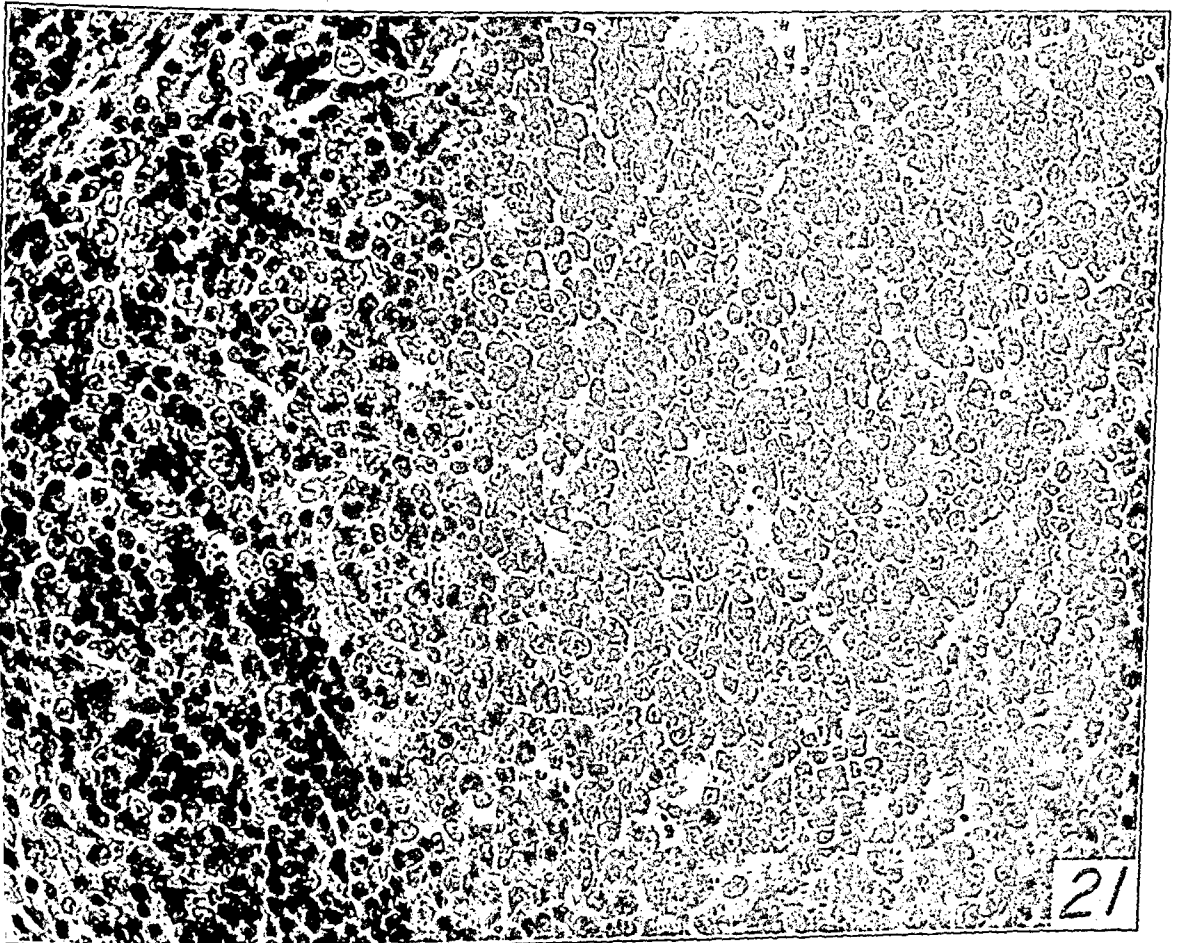
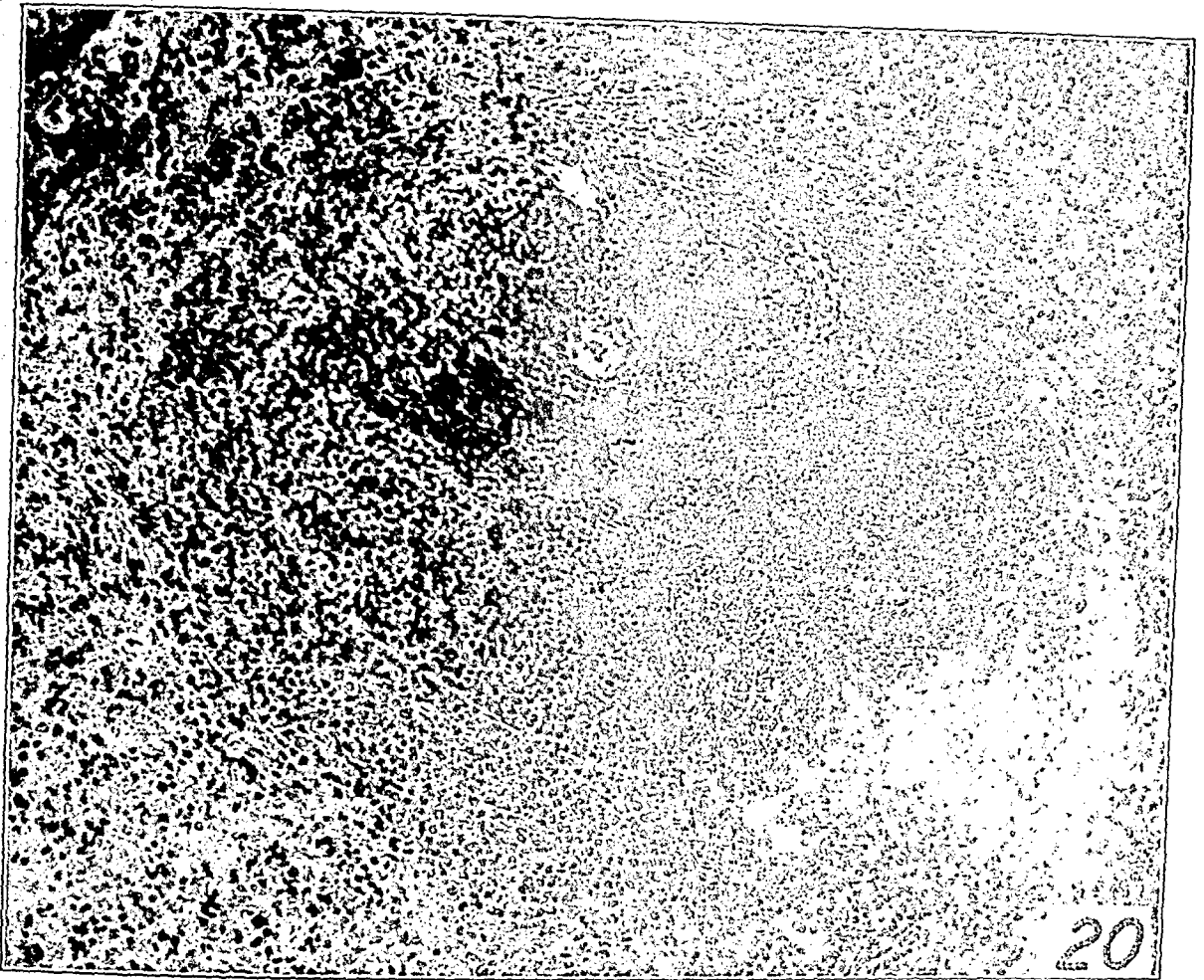


PLATE 140

FIG. 22. Germinal center of spleen 10 days after inoculation of avian type of tubercle bacillus. Note greater hyperplasia than in Fig. 20. Pulp more cellular.  $\times 200$ .

FIG. 23. Higher power of Fig. 22. Note that cells are in large part composed of the larger, paler staining type of cell. Mitoses numerous. Compare with Fig. 21.  $\times 400$ .

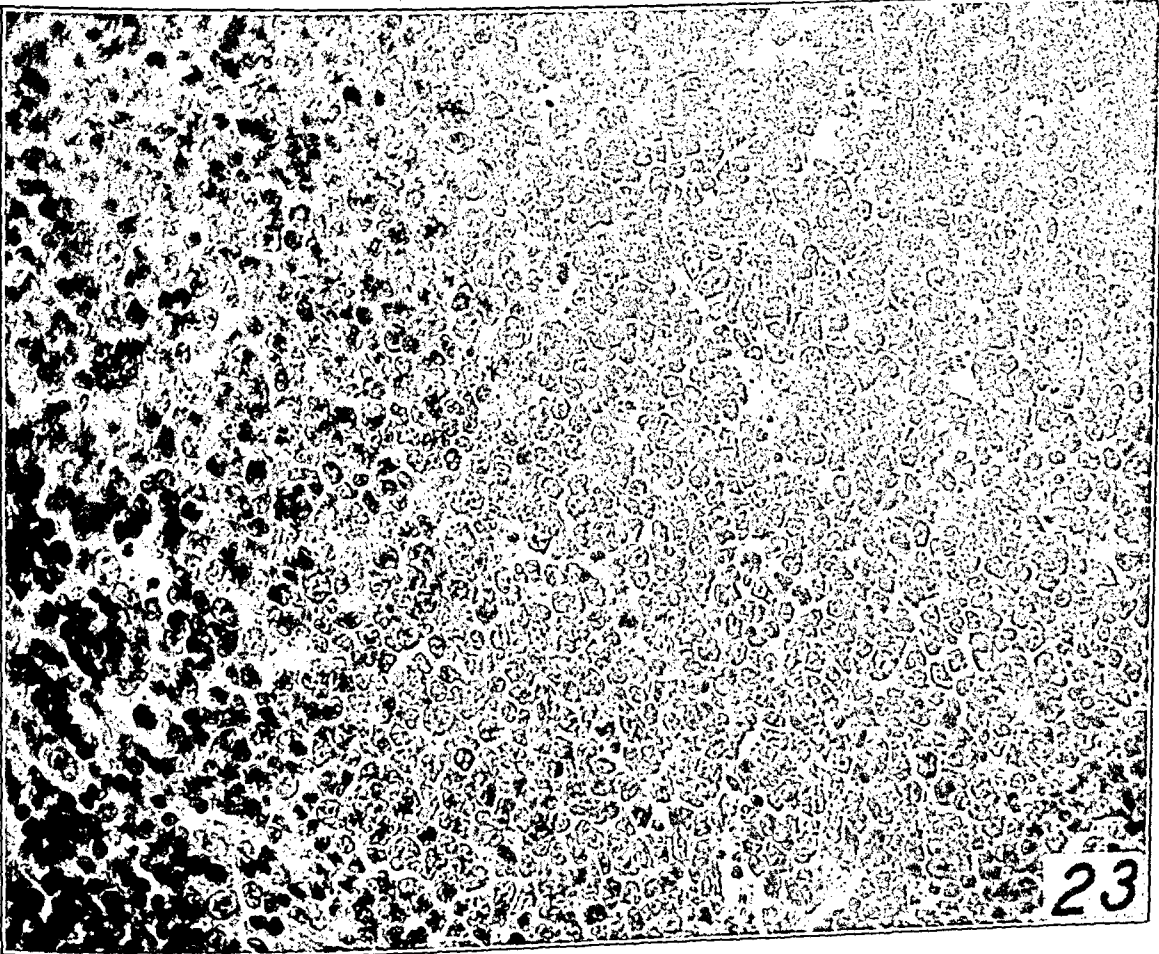
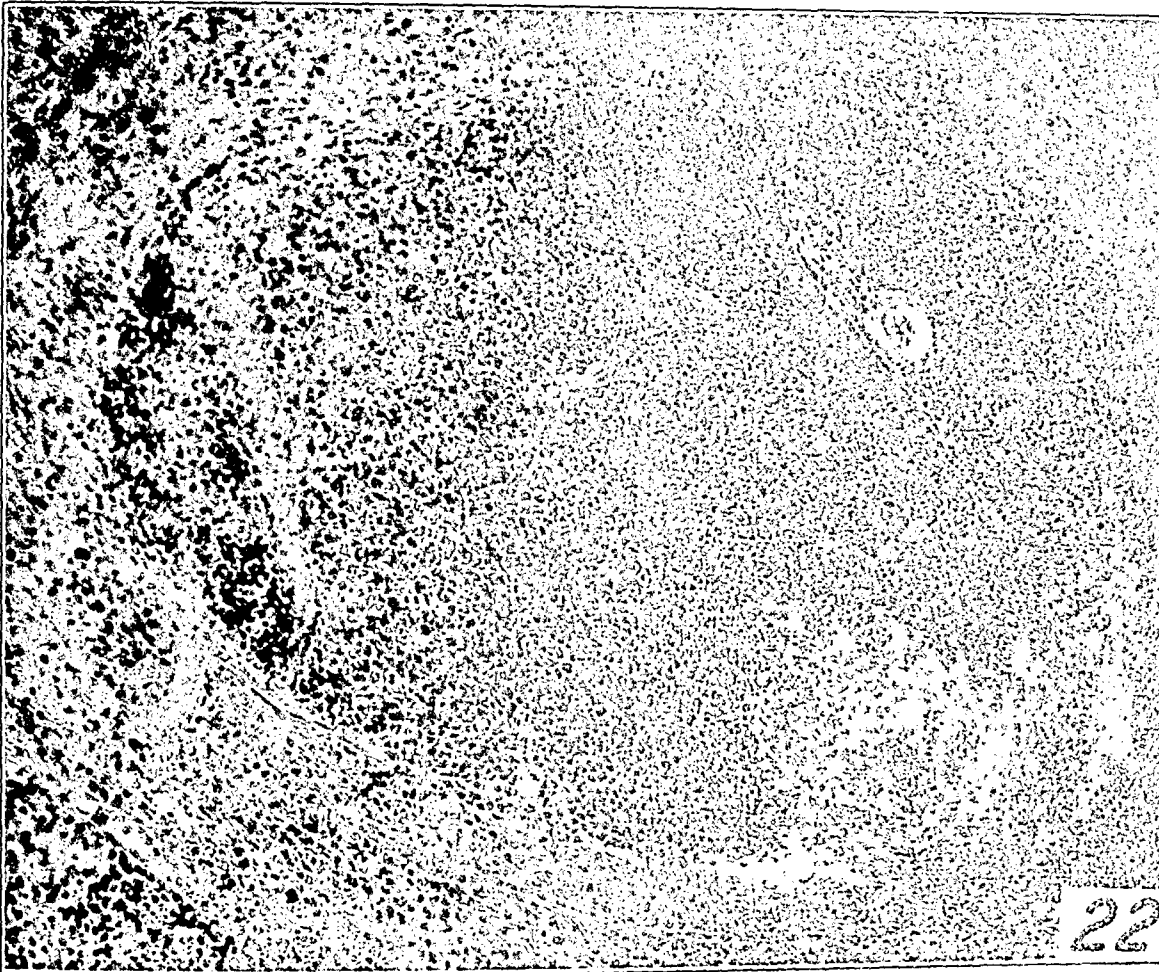


PLATE 141

- FIG. 24. Germinal center and pulp of spleen 14 days after injection of the avian type of tubercle bacillus. Note the large germinal center, the cellular pulp and the monocytic tubercles in the germinal center and the pulp. Compare with Figs. 20 and 22.  $\times 100$ .
- FIG. 25. Higher power from edge of germinal center in Fig. 24. Mitotic figures present. Note admixture of small deeply staining, medium sized paler staining, and large pale staining, typically tuberculous monocytic cells. Compare with Figs. 21 and 23.  $\times 400$ .

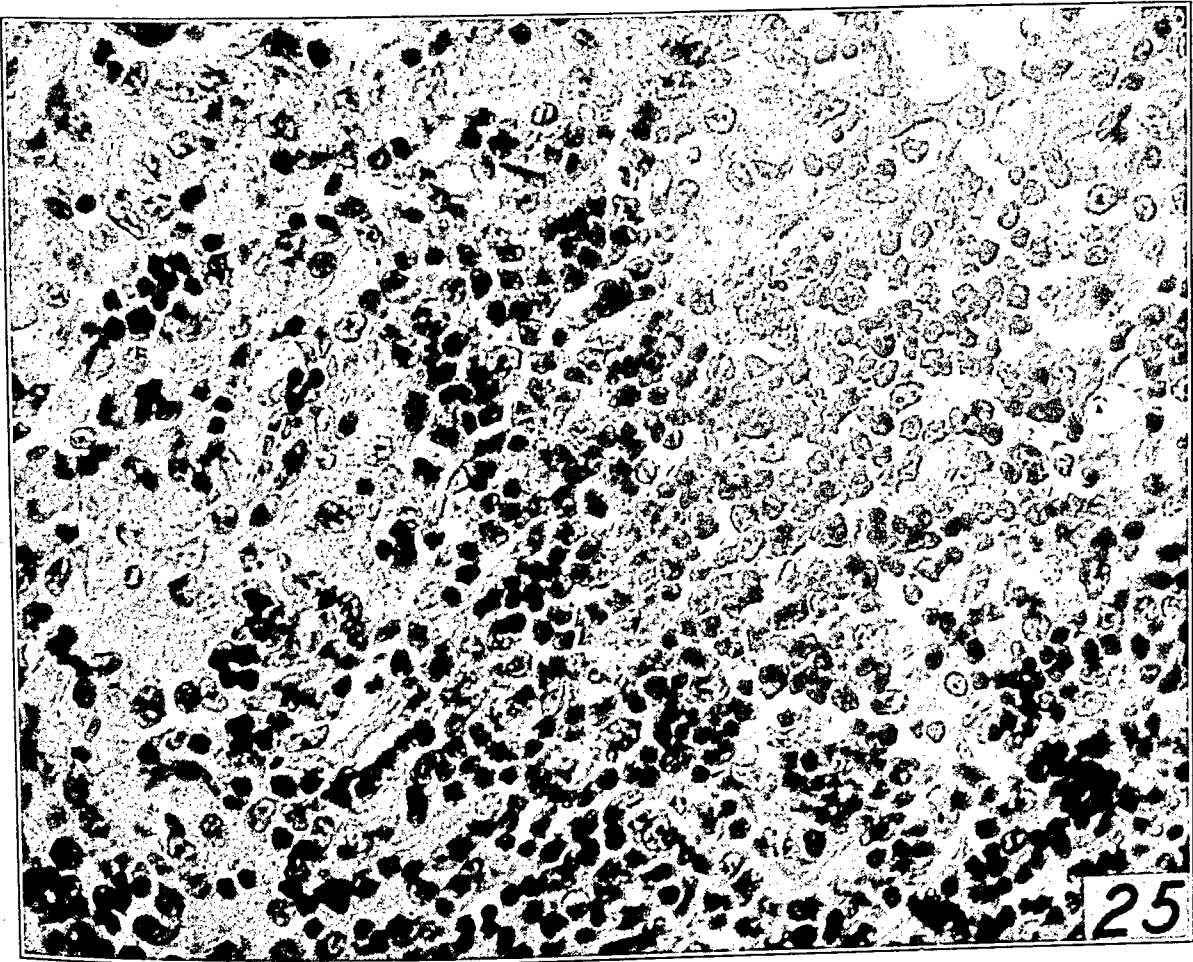
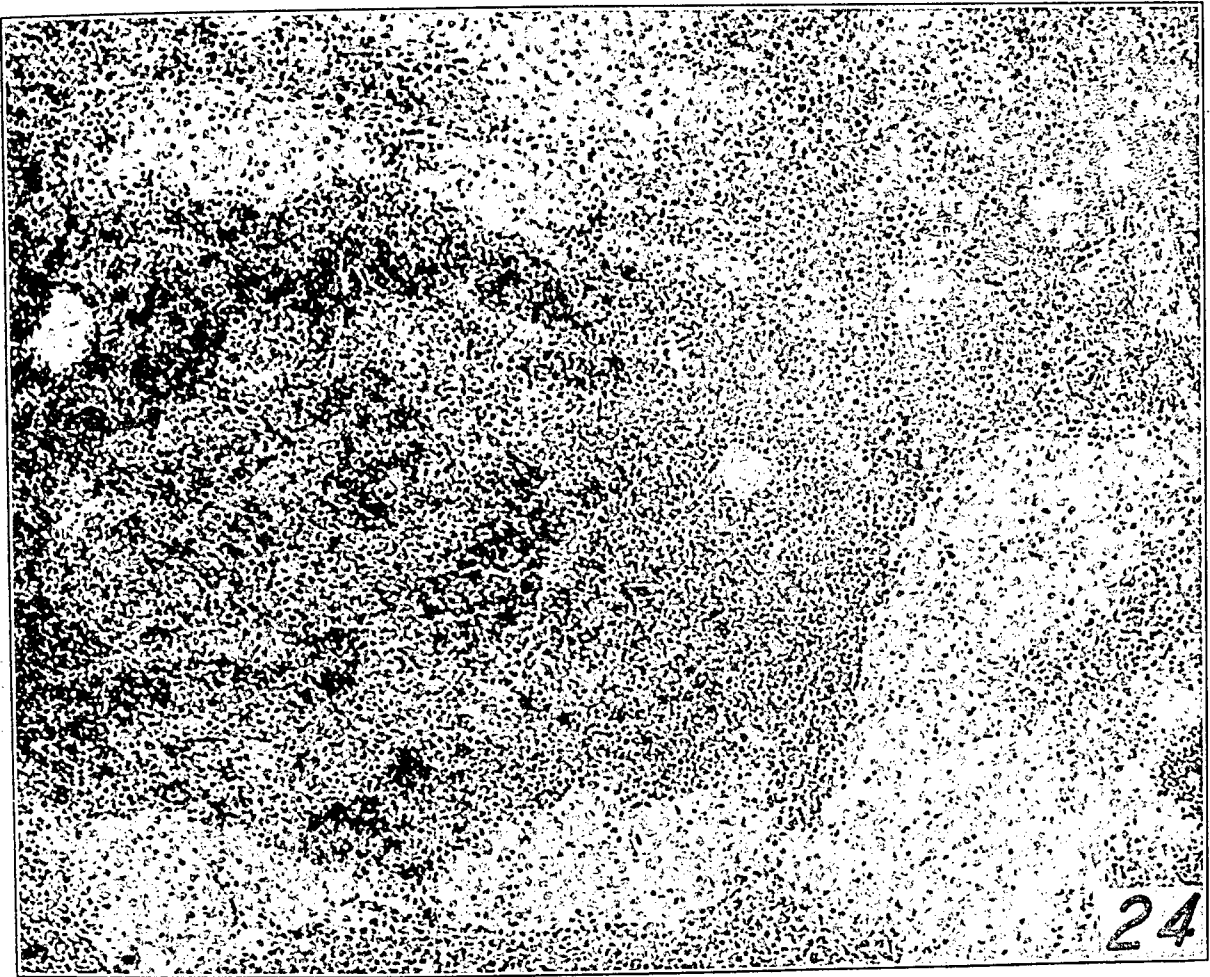


PLATE 142

- FIG. 26. Portion of central part of germinal center in Fig. 17, 3 weeks after inoculation of the avian type of tubercle bacillus. Note the artery, the small number of germinal center cells which remains, and the extensive encroachment of the large pale staining monocytes into the germinal center. Compare with Figs. 21 and 23.  $\times 800$ .
- FIG. 27. Sinusoid from spleen illustrated in Fig. 17. Note normal appearing endothelium at left and two mitotic figures lying free in the blood.  $\times 800$ .
- FIG. 28. Tubercle in pulp from spleen shown in Fig. 17. Note the accumulation of neutrophiles, especially in the central portion. With the death of the neutrophiles and the loss of staining power of their nuclear content the typical picture of caseation occurs.  $\times 800$ .



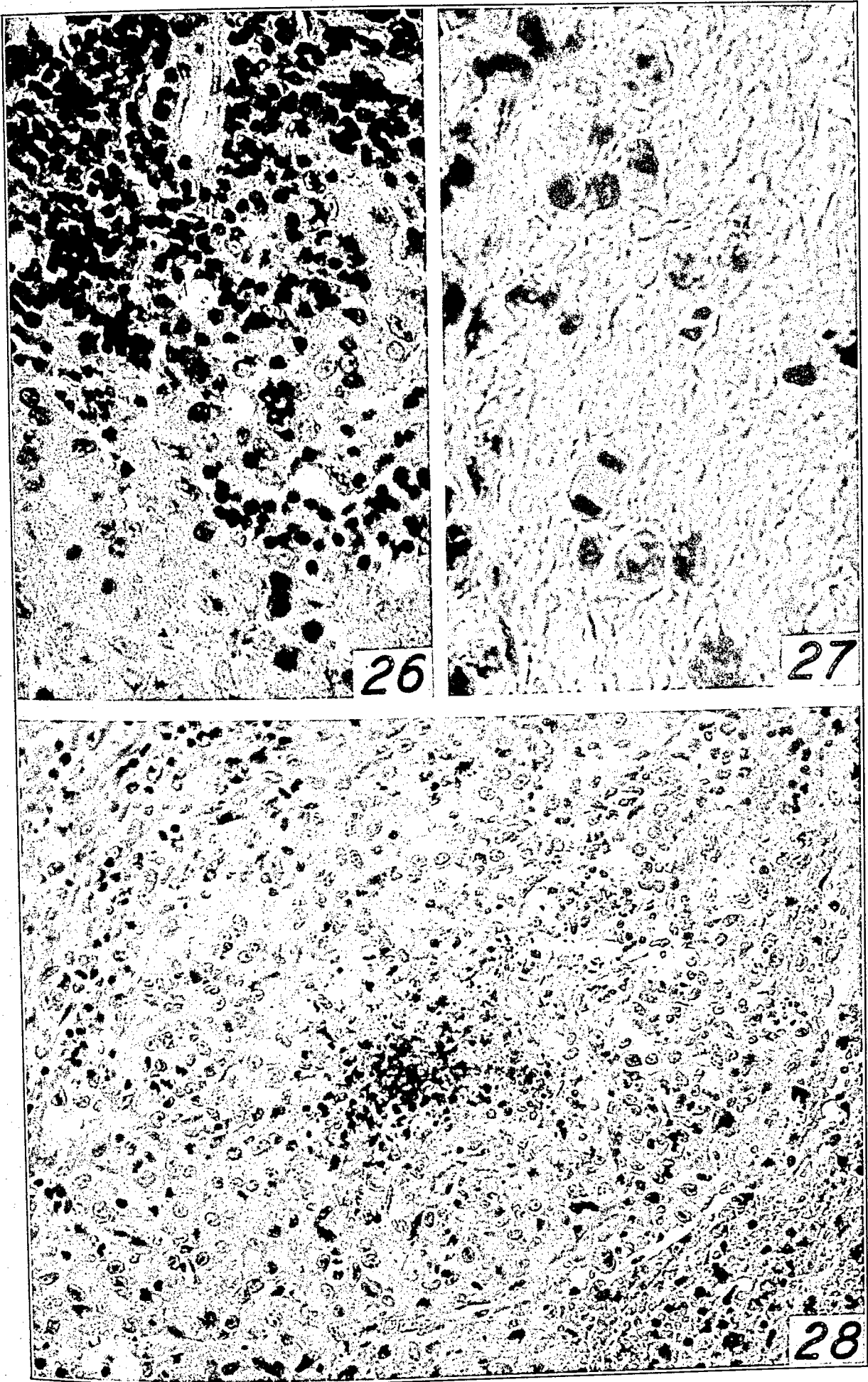




PLATE 143

- FIG. 29. Appendix of normal rabbit. Note the flask shaped lymphoid apparatus with a serosal and mucosal portion. See description in text.  $\times 50$ .
- FIG. 30. Appendix of rabbit 2 weeks after inoculation of bovine type of tubercle bacillus. Compare with Fig. 29. Note great increase in size of serosal portion of lymphoid structure.  $\times 50$ .
- FIG. 31. Appendix of rabbit 3 months after inoculation of bovine type of tubercle bacillus. Note two tubercles in serosal portion and the small serosal and prominent mucosal areas in the lymphoid tissue not infected with tubercle bacilli. Compare with Figs. 29 and 30.  $\times 50$ .

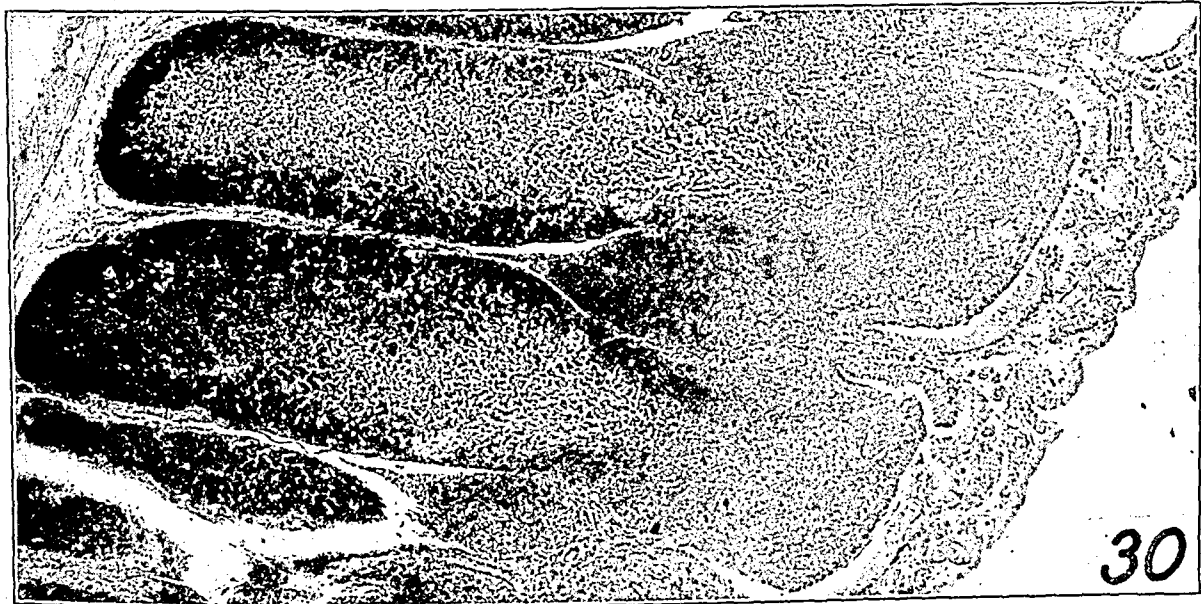
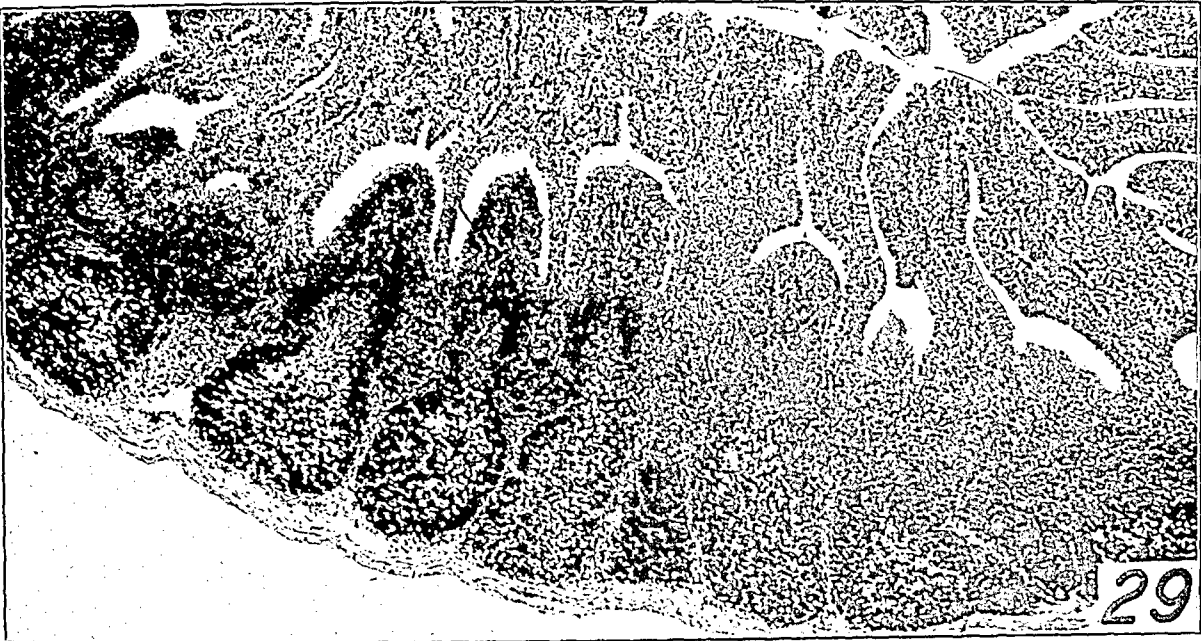


PLATE 144

FIG. 32. Serosal portion of lymphoid tissue from Fig. 29.  $\times 500$ .

FIG. 33. Serosal portion of lymphoid tissue from Fig. 30. Note numerous mitotic figures. Compare with Fig. 32, especially the peripheral, deeply staining area. In Fig. 33 only a part of the peripheral area can be shown because of the marked hyperplasia.  $\times 500$ .

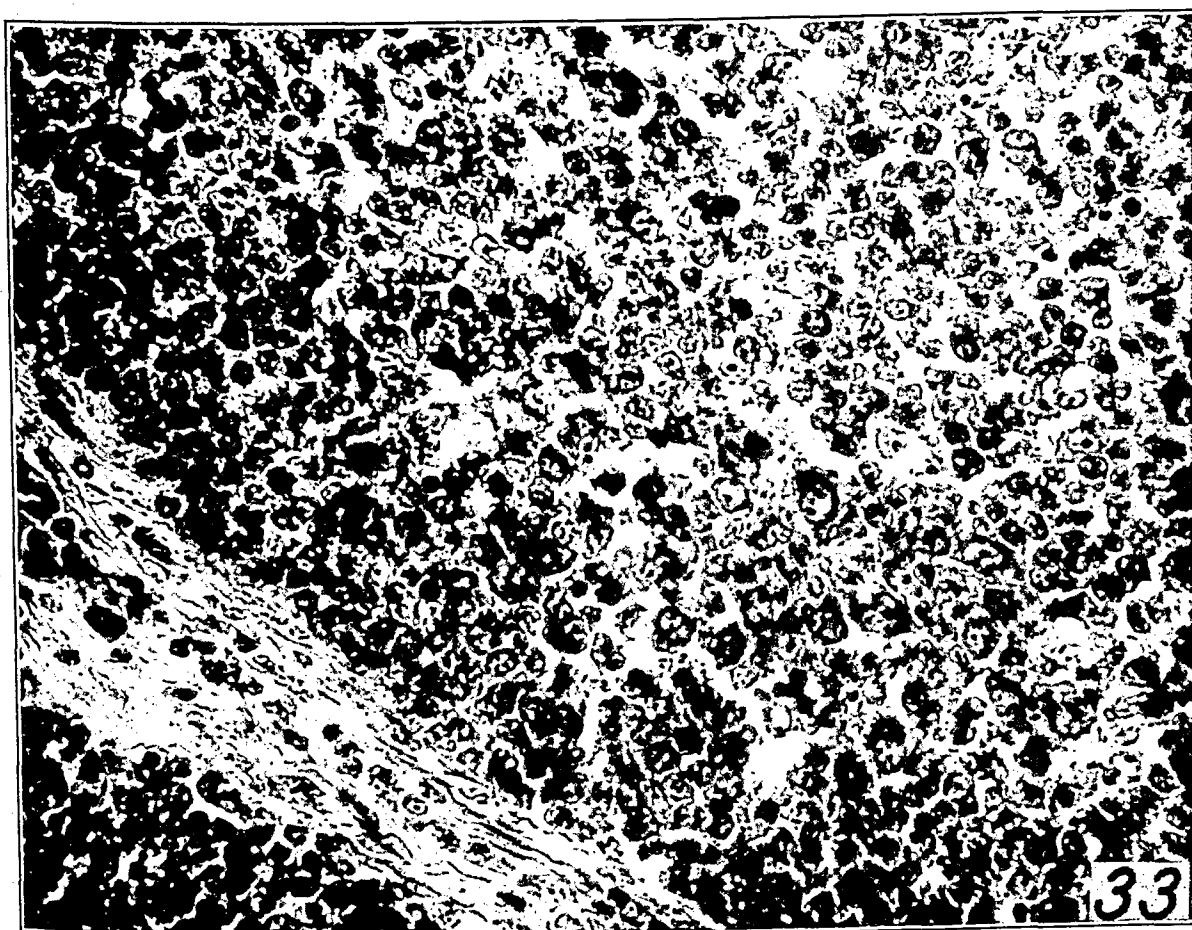
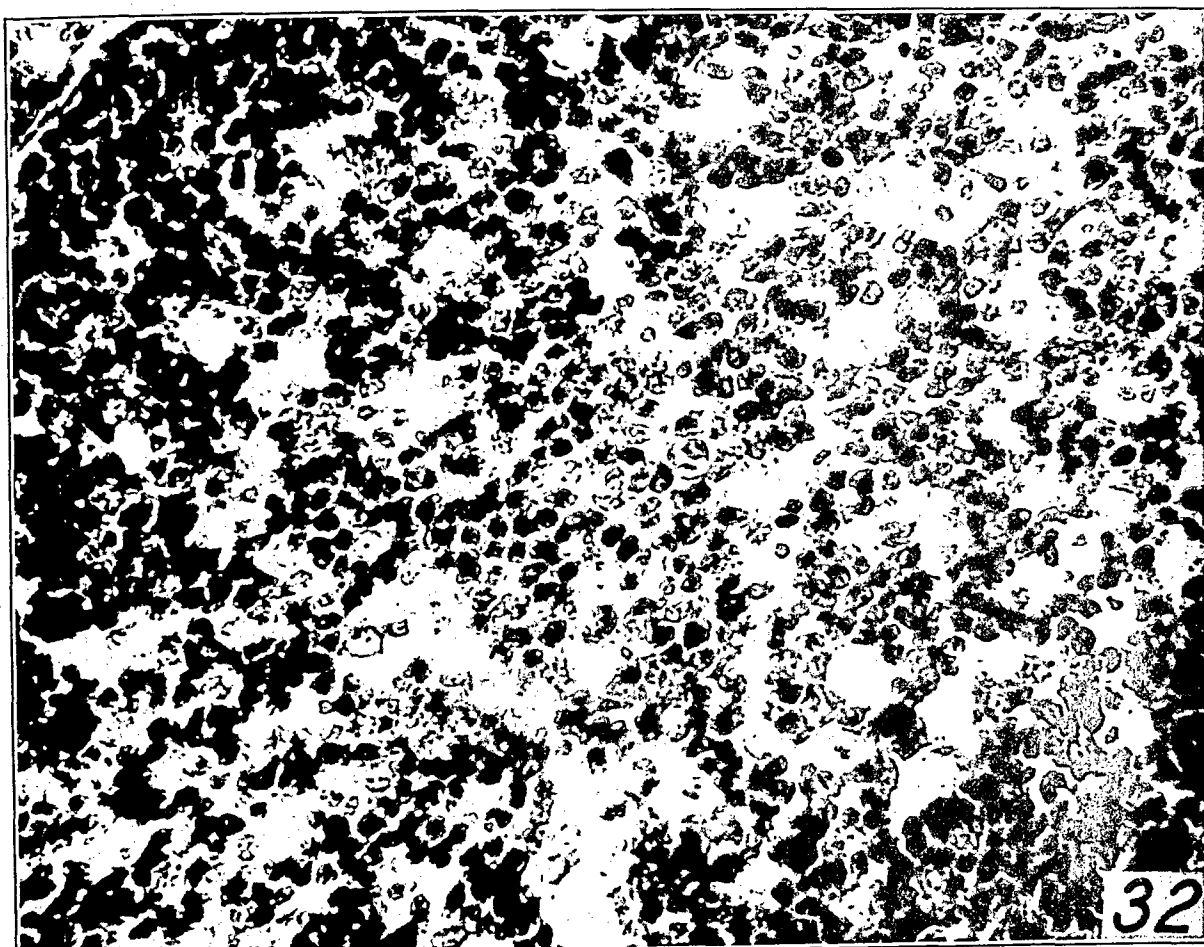
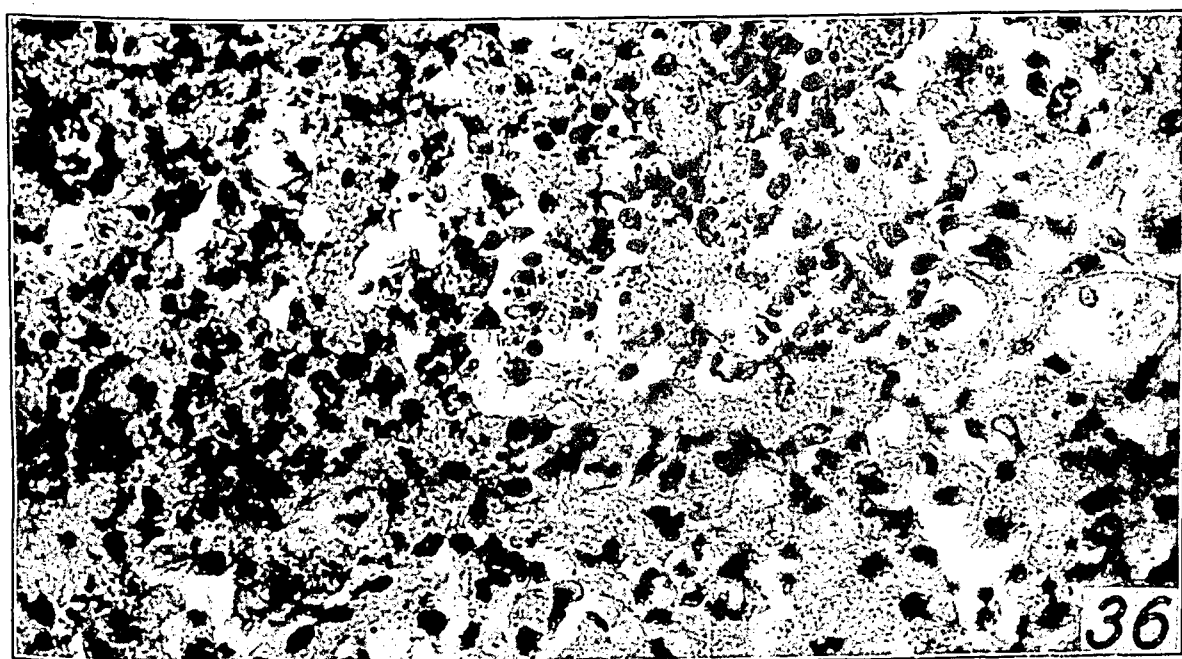
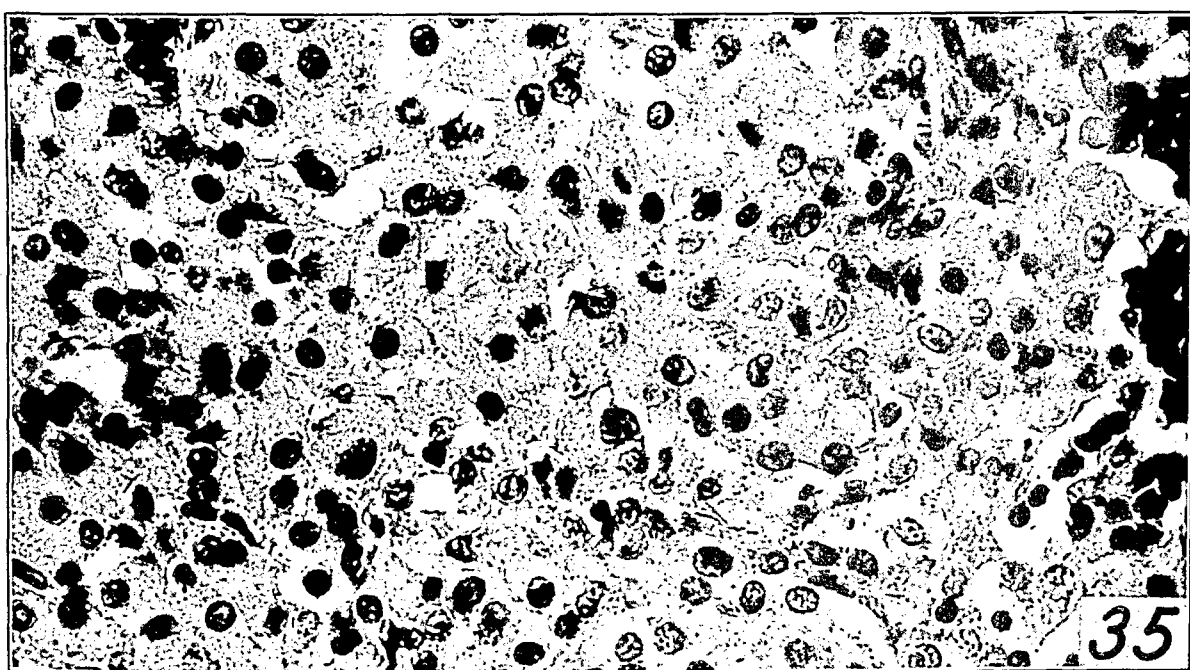
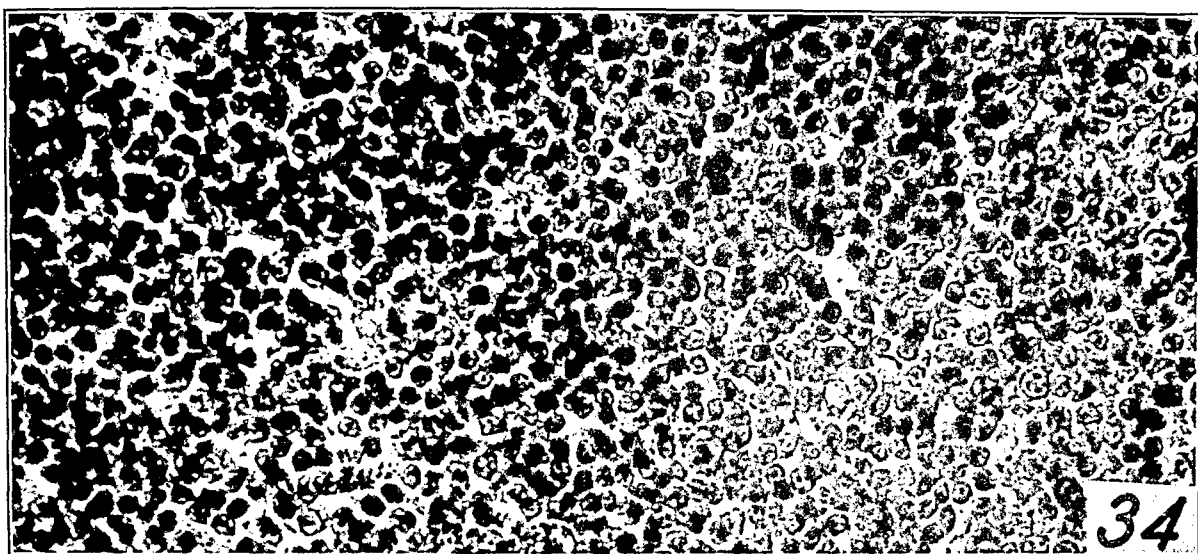


PLATE 145

FIG. 34. Mucosal portion of lymphoid tissue from Fig. 30. Note small uniform size of cells and absence of mitoses. This is more typical of lymphocytic type of tissue. Compare with Figs. 32 and 33.  $\times 500$ .

FIG. 35. Monocytic tubercle from a different area of the appendix shown in Fig. 31. Note the well preserved character of the monocytes and their similarity to the same type of cell shown in Figs. 8, 9, 15 and 28.  $\times 500$ .

FIG. 36. From the same appendix as Fig. 35. An area where the monocytes have largely necrosed and great infiltration of neutrophiles (irregular deeply staining nuclear masses) has occurred — a typical tuberculous abscess. With death of the neutrophiles and disintegration of their nuclei the typical picture of caseation appears as shown in Fig. 31.  $\times 500$ .





## LESIONS OF THE CARDIAC VALVES IN RHEUMATIC FEVER \*

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The early descriptions of valvular disease dealt with the recognition of verrucae and the occurrence of gross valvular deformities. Aside from the absence of microscopic studies these descriptions were of limited value, first because of failure to recognize the importance of rheumatic fever as a cause of valvular disease and later because of confusion of valvular deformities due to rheumatic fever with those due to bacterial endocarditis, arteriosclerosis, syphilis, and so on. In the early part of the eighteenth century, Vieussens<sup>1</sup> gave clinical and pathological descriptions of mitral stenosis, aortic stenosis and aortic insufficiency. In the latter half of the century the thickening, whitening, loss of transparency and ossification of the semilunar valves were mentioned by various writers, including Morgagni,<sup>2</sup> de Senac,<sup>3</sup> and Baillie.<sup>4</sup>

Somewhat later Corvisart<sup>5</sup> described mitral stenosis and warty vegetations on various valves of an apparently rheumatic heart but believed the vegetations to be luetic. Laennec,<sup>6</sup> who termed these vegetations verrucae, and also Bertin,<sup>7</sup> failed to recognize their rheumatic origin but doubted the luetic theory of their formation. While Bouillaud<sup>8</sup> was not the first to observe the occurrence of heart disease in rheumatic fever, he most clearly recognized the association of various valvular lesions with that disease. He emphasized the thickening and stenosis of valves, the occurrence of verrucae at or near the free border rather than at the bases, and the occurrence of stages in valvular disease accompanied by organization and lime infiltration. However, there is considerable evidence that many of his cases were instances of bacterial endocarditis or of degenerative valvular disease. Watson<sup>9</sup> adequately depicted the most striking gross features of rheumatic valvular disease including the thickening, loss of transparency and pliancy, puckering, adhesions and vegetations.

During the latter half of the nineteenth century a number of reports dealt with the microscopic findings in rheumatic valvular disease. While the significance of bacteria in the causation of endo-

\* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.

Received for publication March 30, 1936.



carditis was understood, inadequate or imperfect bacteriological technique, incomplete clinical knowledge of the bacterial forms of endocarditis, uncertainty as to the bacterial etiology of rheumatic fever and the absence of rigid pathological criteria for the latter were some of the more important reasons for confusion. In the reports of Jaccoud,<sup>10</sup> Crocker,<sup>11</sup> Weichselbaum,<sup>12</sup> Klebs,<sup>13</sup> Birch-Hirshfeld,<sup>14</sup> and others, bacterial and rheumatic endocarditides were confused. The division of endocarditis into verrucous and ulcerative forms was not clear-cut because the former included bacterial as well as rheumatic lesions. Wyssokowitsch<sup>15</sup> and Orth<sup>16</sup> most clearly recognized that many of the cases of verrucous endocarditis showed no bacteria in the vegetations. The latter author stressed the importance of cellular proliferation in the valve and the avascularity of normal valves.

Detailed microscopic studies of valvular lesions in rheumatic endocarditis were reported by Achalme,<sup>17</sup> and by Königer,<sup>18</sup> and the formation of verrucae was discussed by Neumann,<sup>19</sup> and Ziegler.<sup>20</sup> The first mentioned reported bacterial infiltration of the valves and described what he considered to be the causative organism. Neumann conceived verrucae as being formed purely from the valve substance which had undergone fibrinoid degeneration. Ziegler, on the other hand, considered the verrucae to result from marantic thrombosis with deposition on the valve surface (thrombo-endocarditis). Königer's painstaking histological studies were based essentially on valvular disease in non-rheumatic infections. Only 2 definitely rheumatic cases were presented. The primary process was described as an endothelial necrosis. Verrucae were considered to be formed from the subendothelial tissues which proliferated, underwent coagulation necrosis and combined with thrombotic material deposited from the blood stream.

Later studies were characterized by a clearer delimitation of rheumatic endocarditis from subacute and acute bacterial endocarditis, from syphilitic disease and from arteriosclerotic valvular disease, especially of the Mönckeberg variety. This segregation resulted from the recognition of the Aschoff body as a specific criterion of rheumatic disease, from improved blood culture methods and more detailed clinical studies of bacterial endocarditis, from the discovery of the Wassermann reaction, and finally from more thorough knowledge of the histology of the normal valve. Nevertheless, there was still confusion between rheumatic and non-rheumatic cases in the

reports of Dewitzky<sup>21</sup> and of Felsenreich and von Wiesner.<sup>22</sup> The former, however, gave clear descriptions of the chronic valvular lesions in rheumatic fever and segregated them from those of Mönckeberg's ascending sclerosis of the aortic valve. The latter authors recognized some of the characteristic alterations of the elastica, and the lesions in the pockets of the valves and chordae tendineae attachments.

To Bulloch,<sup>23</sup> and especially to Carey Coombs,<sup>24</sup> we owe most of our recent knowledge of the histology of rheumatic valvular disease. Both of these authors, as well as Butterfield,<sup>25</sup> Thalheimer and Rothschild,<sup>26</sup> Gengenbach,<sup>27</sup> Clawson and Bell,<sup>28</sup> and others, described the presence of Aschoff bodies in the valves. Bulloch emphasized the occurrence of the earliest changes, not in the endothelial but in the subendothelial layers. These consisted of a coagulation necrosis with swelling and homogeneous transformation of the ground substance. Coombs drew attention to the proliferative and exudative reactions in the valves as well as in other regions of the heart. The proliferative reaction was the more marked. The deeper structures reacted before there was evidence of injury to the endothelial surface. This observation, among others, led to the concept of a deep valvulitis first and verrucous formation later. Following Poynton and Paine,<sup>29</sup> Coombs believed that infection came by way of the coronary branches and not superficially from the circulating blood. Like Poynton and Paine he also described what he believed were bacteria in the valve.

Similar descriptions were given in the more recent studies of Clawson, Bell and Hartzell,<sup>30</sup> and by Klinge<sup>31</sup> and his coworkers. The former authors described acute and healing lesions in rheumatic endocarditis and old valvular defects. Klinge described an acute stage of valvular inflammation with subendothelial focal and banded swellings, a later granulomatous stage with Aschoff bodies or diffuse cellular infiltrations, and finally a stage of scarring. The valvular lesion was considered primary and the verrucae secondary.

The detailed description of valvular lesions given by Ribbert<sup>32</sup> is essentially like that of Königer. Benedict<sup>33</sup> gave a differential pathological description between rheumatic and syphilitic disease of the aortic valve. He particularly emphasized that in lues there was an increase of endocardial elastica, whereas in rheumatic fever the elastica was torn and diminished in amount. Leary<sup>34</sup> drew attention

to palisade cell formations along the contact edges of the valves which he believed represented a specific early rheumatic reaction. Of the 3 cases which he reported, only 1 was definitely rheumatic, and that patient died of an acute infection.

Many of the more recent studies have concerned themselves with investigating grossly normal valves from patients dying of acute infections, or the grossly normal portions of valves which elsewhere showed macroscopic abnormality. Such reports were made by Baldassari,<sup>35</sup> Holsti,<sup>36</sup> Böhmig and Krückeberg,<sup>37</sup> de Vecchi,<sup>38</sup> and by Waldow.<sup>39</sup>

In summarizing and appraising the above mentioned reports on the pathogenesis of rheumatic valvulitis, they may be divided into two periods. During the period preceding the discovery of the Aschoff body as the specific lesion in rheumatic fever, the reports were largely confused by the failure in many cases to differentiate the valvular lesions of rheumatic fever from those occurring in other endocarditides as well as from other degenerative valvular changes. Following the discovery of the Aschoff body confusion still persisted: (1) because this lesion is not invariably present in rheumatic fever, especially during the less active stages; (2) because the unfortunate classification of endocarditis into verrucous and vegetative led to no sharp differentiation of the several types; (3) accurate descriptions of the normal structure of valves were not available, with the result that the pathological changes were seldom referred to with precision in respect to the layers of the valve involved; and (4) age period changes occurring in normal valves received practically no attention, thus adding considerably to the difficulty of discerning the lesions due essentially to the chronic rheumatic process.

In a series of studies reported by one of us (L. G.) with collaborators, attention was drawn to the pathogenesis of various rheumatic lesions occurring in the heart, *viz.*, of the myocardium<sup>40</sup> (Aschoff bodies), blood vessels,<sup>41</sup> large vessel roots,<sup>42</sup> auricles,<sup>43</sup> conduction system,<sup>44</sup> pericardium<sup>45</sup> and valve rings.<sup>46</sup> In these investigations a description of the normal histology and topography as well as of the age period changes in these sites was included. In following the life cycle of these lesions in indisputable cases of rheumatic fever certain stigmata of active as well as healed rheumatic lesions were demonstrated. These stigmata of healed rheumatic lesions are particularly

important in establishing the essential rheumatic nature of a given valvular lesion. On the basis of such studies it now becomes possible sharply to define rheumatic from non-rheumatic hearts, even in the absence of Aschoff bodies or a typical clinical history.

According to the definitions of Gross and Kugel,<sup>47</sup> the auriculo-ventricular valve leaflet consists of the fibro-elastic structure immediately distal to the auricular myocardial wedge, and the semi-lunar valve leaflet consists of the fibro-elastic structure attached to the subjacent ventricular myocardium through the intermediary of annulus interdigitations (Figs. 1 and 2). This definition includes the valve ring as the proximal portion of the leaflet.\* The ring lesions in rheumatic fever recently described by the present authors, therefore, can be legitimately considered as part of the valvular lesion as a whole. These rheumatic ring lesions have been described separately for purposes of clarity, but reference should be made to the complete report in order to preserve a logical continuity with the descriptions to be given of the lesions in the remainder of the leaflet.

Briefly to recapitulate the findings in non-rheumatic and rheumatic rings, the following points should be borne in mind: Normal valve rings are practically devoid of inflammatory cells. The ring spongiosa in the semilunar cusps almost invariably consists of a gelatinous tissue and is generally sharply separated from the adjacent fibrous structure (annulus). In the auriculoventricular valves the spongiosa component is generally inconspicuous. Blood vessels with muscular walls are never seen in the normal rings. The incidence of capillaries in these rings, as determined in 100 normal hearts, is as follows: anterior mitral valve ring, 1 per cent; posterior mitral valve ring, 2 per cent; aortic valve ring, 0 per cent; tricuspid valve ring, 14 per cent; and pulmonary valve ring, 7 per cent. When present, the capillaries are generally few in number, small and circular on cross-section, and in structure easily differentiated from granulation tissue capillaries.

On the other hand, the characteristic features of the ring lesions in rheumatic fever are briefly as follows: The rings are almost invariably infiltrated with inflammatory cells, capillaries and blood vessels. The latter are sometimes of a characteristic type. The inflammatory process generally spreads into the contiguous valve leaflets

\* A full description and delimitation of the valve rings were reported by Gross and Kugel.<sup>47</sup>

as well as along the annulus extensions of the aortic root. These contiguity extensions of the inflammatory process are present in the septum fibrosum as well as in the intervalvular fibrosa (the collagenous link between the aortic and mitral valves). The subvalvular angles show characteristic lesions, termed reduplications. These are frequently inflamed and vascularized. Scarring of the ring occurs, with obliteration of the ring spongiosa. The extent of these inflammatory phenomena is determined by the clinical course of the disease.

Bearing in mind, therefore, this intimate relation of the ring lesions to those that occur in the remainder of the valve leaflets in rheumatic fever, we are now in a position to take up the description of the gross and microscopic changes that take place in the latter and to discuss their significance with regard to the pathogenesis of rheumatic valvulitis. The description of these lesions will be preceded by a discussion of the gross and microscopic findings in normal valves, together with a consideration of their age period changes.

#### MATERIAL AND METHODS

The material consisted of 40 non-rheumatic control hearts and 97 rheumatic hearts. Seventy-one of the latter were from active rheumatic cases and showed Aschoff bodies in the myocardium, and 26 showed chronic valvular disease of the typical rheumatic variety but without evidence of activity either clinically or pathologically and with no demonstrable Aschoff bodies in the myocardium. The grouping as to activity and inactivity was based on the criteria outlined by Rothschild, Kugel and Gross.<sup>48</sup> Particular care was taken to avoid material that in any way indicated the possibility of a co-existing bacterial endocarditis or syphilis. A careful study of the clinical records and pathological specimens made it possible to divide the rheumatic cases into the following groups:

- GROUP I. Active cases where death took place during the first attack (12 cases).
- GROUP II. Active cases where one preceding attack occurred within 1 year of the fatal outcome (7 cases).
- GROUP III. Active cases where one previous attack occurred at least 2 years previous to the fatal outcome (11 cases).

GROUP IV. Active cases with a history of repeated attacks, death occurring during an acute recurrence (13 cases).

GROUP V. Active cases where death was caused by decompensation without clinical evidence of a final acute attack. In some of these cases there was no previous history of rheumatic fever (28 cases).

GROUP VI. Inactive cases of chronic valvular disease of the typical rheumatic variety (26 cases).

The sections from which these studies were made were cut according to the standardized technique of Gross, Antopol and Sacks,<sup>49</sup> and the technical procedures were those previously described by Gross and Ehrlich.<sup>40</sup>

#### AGE PERIOD CHANGES IN THE GROSS APPEARANCE AND HISTOLOGICAL STRUCTURE OF NORMAL VALVES

A study of the gross appearance of normal valves revealed only such alterations as could be ascribed to increasing age and tension. In about half of the cases, particularly in the older age periods, the uniform slenderness and transparency of the valve cusps were slightly altered by the occurrence of isolated patches of whitish opaque thickening. These thickenings were generally not notable. They were situated at various portions of the valve leaflets but most frequently at the closure line and free margin. When present at the free margin, the thickening obscured the normal concave scalloping and rendered the margin either straight or convex.

Not infrequently there was broadening of the heads of the chordae tendineae that were attached to the region of valvular thickening. In addition to these, there were occasional yellowish lipid flecks which spotted the valve ring, both aspects of the valve cusps, and especially the valve pockets.

The pocket of the normal valve generally formed a sharp narrow angle which was traversed only occasionally by an isolated bridge of fibrous tissue. Except for the lipid flecks just mentioned, there were none of the irregularities that will be described as occurring in the pockets of the various rheumatic valves.

The histological structure and topographical relations of normal human heart valves have been described in detail by Gross and

Kugel.<sup>47</sup> Briefly considered, they are characterized by the following features: All valve leaflets carry as their main backbone a dense collagenous layer called the fibrosa (Figs. 1 and 2). Adjacent to the fibrosa layer and sometimes not clearly distinguished from it, there is a zone of loose connective tissue called the spongiosa layer. This is situated on the auricular aspect of the auriculoventricular valve fibrosa as well as on the ventricular aspect of the semilunar valve fibrosa. In the semilunar cusps the spongiosa layer may be so conspicuous as to constitute a sharply defined zone of loose gelatinous tissue. In the auriculoventricular cusps the spongiosa layer is frequently quite inconspicuous in its proximal two-thirds and becomes discernible and widened generally only in the presence of inflammation. The distal third, or tip of the valve, generally consists of a gelatinous expansion of the spongiosa layer. On the auricular surface of the auriculoventricular leaflets there lies a fibro-elastic mantle of various thickness, called the auricularis layer. This is covered by a flat layer of endothelial cells. The ventricular surface of these leaflets is covered by a much thinner layer of fibro-elastic tissue called the ventricularis. This, in turn, is also covered by endothelium. The ventricular aspect of the semilunar cusps is clothed by a fibro-elastic mantle somewhat more delicate than the auricularis of the auriculoventricular cusps. This is called the ventricularis of the semilunar cusps. The arterial aspect of the semilunar cusps is covered by an even more delicate elastic mantle. Inasmuch as the auricularis layer of the auriculoventricular valves and the ventricularis layer of the semilunar valves are the first to be impinged by the blood stream, these will be referred to as the "proximal layers." For similar reasons, the ventricularis layer of the auriculoventricular valves and the arterialis layer of the semilunar valves will be referred to as the "distal layers." These terms are not to be confused with the proximal and distal portions of the valves, *i.e.*, the insertions and tips, respectively.

Normal valve leaflets are poor in cells. This is particularly noticeable in the fibrosa layer. In spite of the controversial reports on the existence of blood vessels in valves, the available evidence leaves little room for doubt that, apart from the sparse capillaries mentioned above as occasionally occurring in the valve ring, blood vessels are seldom, if ever, present in normal human heart valves.<sup>50</sup> It may be stated in passing that the high incidence of valve vascularization,

as recently reported by Wearn *et al.*,<sup>51</sup> can be adequately accounted for chiefly by the inclusion into the statistics of vessels supplying the auricular myocardial wedges of the auriculoventricular valves, the occasional presence of ring capillaries, and acceptance of extinct or mild valvulitides as normal material.

With advancing age periods, the various layers of the valves become progressively poorer in cells and take on the following changes: The strata become increasingly well defined; the semilunar cusp spongiosa becomes more and more fibrous and elastified; the auriculoventricular valve ring spongiosa and the spongiosa situated opposite the chordae tendineae insertions become loose and often the seat of fat deposits; the elastic membranes become heavier and longer; the auricularis and often the ventricularis layers become appreciably denser, more collagenous and thickened; and the collagenous fibrosa undergoes degenerative lipoid changes. The point last mentioned is inevitably associated with the calcium salt deposition of the later age periods.

The tips (distal portions) of the valve leaflets become somewhat thickened with advancing age periods. In the auriculoventricular valves, particularly the mitral, this thickening is due to two processes, *viz.*, fibro-elastification of the auricularis layer at the closure line, and absorption of thickened chordae tendineae insertions into the fibrosa. The fibro-elastified thickening at the site of the closure line is generally quite dense, somewhat oval on cross-section and never contains inflammatory cells or blood vessels. The chordae tendineae insertions beneath the tips of the leaflets show reduplications of their endocardial covering. These may become so exaggerated and agglutinated to one another as to thicken appreciably the tip of the cusp. As will be shown subsequently, however, thickening at the tips of rheumatic valves is due to an entirely different process.

Other age period changes in the auriculoventricular cusps are the formation of delicate crescentic reduplications of the ventricularis layer around the insertions of the chordae tendineae of the second and third order. In the semilunar cusps advancing age produces a more gradual and more delicate elastification of the ventricularis layer, particularly near the semilunar folds. The noduli Arantii and Morgagni become markedly elastified and hyalinized. Here again inflammatory phenomena are absent.



## GROSS APPEARANCE OF RHEUMATIC VALVES IN GROUP I

*(12 Active Cases Where Death Took Place During the First Attack)*

The most frequent gross alteration of the valve leaflets observed in this group was a definite diffuse thickening. This was invariably present in the mitral and aortic valves and in eight of the twelve tricuspid valves, but the pulmonic valves, with one exception, appeared normal. In general, the thickening was uniform throughout the cusp, but in the mitral valve this change was accentuated at the closure line by the formation of a fine ridge.

The normally concave, scalloped, sharp margins of the auriculo-ventricular valves were almost invariably thickened and straight. In about one-third of the mitral valves the peripheral portion of the leaflet became slightly protuberant to form an overhanging shelf.\* In a few of the tricuspid valves the scalloped concavity of the free margin was likewise obliterated, but in no instance was there shelf formation.

The auricular surface of the auriculoventricular valves occasionally showed an irregular corrugation. In 3 cases moderate gross vascularization was noted on the superficial aspects of the auricular surface of the mitral valve and once on the tricuspid valve.

In the aortic valve the sharp margin of the cusps frequently became thickened and rounded, and in about one-third of the cases this rolled margin was slightly inverted toward the sinus pocket. In 2 cases the semilunar folds were elevated toward the free margin, and once the free margin showed a distinct notch at about the site of the nodulus.

Verrucae were present on all of the mitral valves, on eight of the aortic and on seven of the tricuspid. The verrucae were fine, pinhead-sized, yellowish and gray elevations which usually fused with each other. Many of them were fresh; some showed evidences of healing. The verrucae were situated at the closure line, free margin, or at both sites. Extension of the verrucae from the mitral leaflets to the insertions of the chordae tendineae was frequent. They occasionally extended around the free margin to the ventricular aspect of the

\* The term "shelf" is employed to indicate the projection of valve tissue over the first order chordae tendineae insertions in such a manner as to overhang the latter. In contradistinction to the "shelf," prominence of the closure line is referred to as a "ridge."

valve. In 1 case there was a fresh verrucous deposit in the pocket of the posterior mitral cusp. The verrucae tended to form conglomerate mounds on the noduli Arantii of the aortic valve and from there extended in rows along the semilunar folds. Frequently the verrucae occurred in isolated fashion, affecting only a single cusp of a valve or even only part of a cusp. In one instance the verrucae on the tricuspid valve extended onto a papillary muscle.

The pockets of the valves in this group were generally normal. In 1 case, as has been mentioned, there were fresh verrucae in the posterior mitral pocket. In 2 cases there were a few irregular folds and ridges in the sinus pocket of the aortic valves.

In about one-third of the cases in this group there were distinct abnormalities of the chordae tendineae. The occurrence of verrucae at their attachments to the valve has already been mentioned. In 1 case there were organizing verrucae extending halfway down the chordae. Not infrequently the chordae tendineae were distinctly thickened at their attachments to the valves (ham shaped), particularly where these attachments were close to confluent verrucae on the valve. Sometimes they were shortened and rarely was there fusion of isolated chordae. In 1 case the chordae tendineae of the septal leaflet of the tricuspid valve were agglutinated to the underlying endocardium (Fig. 3).

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVES IN GROUP I

The thickening of the valve leaflets in this group was due to inflammation, edema and hypercapillarization of the proximal layers of the valve (auricularis layer of the auriculoventricular valves and the ventricularis layer of the semilunar valves) together with similar involvement of the spongiosa layer (Figs. 4 and 5). The lesions obviously represented a contiguity process from the ring and generally extended along the entire length of the leaflets. The vascularization of these layers, as well as the others to be described, consisted almost entirely of capillaries. Occasionally vessels with muscular walls were noted. These were sometimes of the intimal musculo-elastic hyperplastic type. The inflammatory cells were chiefly lymphocytes. In some cases, however, polymorphonuclear leukocytes predominated. Plasma cells, fibroblasts, macrophages and other mononuclear cells were occasionally seen.

Besides edema, hypercapillarization and exudate, the spongiosa

layer sometimes showed elastica condensation and disruption. Eosinophilic swelling of the collagen was not infrequently seen. This generally involved the spongiosa layer in its main body as well as at the tip of the leaflets. The tip was rarely scarred.

A prominent feature of this group was inflammatory involvement of the fibrosa layer (Figs. 4 and 5). This was greatest in the aortic valve and occurred chiefly in the zone that is adjacent to the spongiosa. It consisted of capillarization, inflammatory cell involvement and sometimes elastic tissue formation. In a number of cases the fibrosa layer contained large swollen cells with basophilic cytoplasm between the collagen bundles. These cells not infrequently bore a resemblance to those that form the Aschoff body. Indeed, in a number of instances, typical Aschoff bodies were seen in the fibrosa layer. This occurred most frequently in the tricuspid and pulmonic valves.

In most of the auriculoventricular valves the ventricularis layer was thickened, inflamed and vascularized. The arterialis layer of the semilunar cusps was also frequently thickened and somewhat inflamed. Vascularization of this layer, however, was infrequent.

The tips or distal portions of the valves in this group generally retained some of their normal spongy structure, even though many of them were the seat of inflammatory lesions including Aschoff bodies. By contrast it will be shown that in the groups to be described, the mitral, aortic and tricuspid valve tips showed increased fibrosis.

In discussing the incidence of verrucous lesions in all the clinical groups studied, as determined by microscopic examination, reference will be made only to those that were fresh, *i.e.*, still possessed eosinophilic hyaline material with or without evidence of organization. The sections cut were generally selected with a view toward including such lesions. Furthermore, as is to be expected, verrucous lesions were sometimes noted microscopically when they were overlooked on gross examination. As a consequence, there will be a discrepancy in the incidence of these lesions as listed under the gross and histological findings, respectively. The latter undoubtedly present a more accurate picture of the actual incidence of those lesions that still contained unorganized verrucous material.

Histological studies on the nature of these verrucous lesions suggest that neither platelets nor fibrin are concerned in their formation. They appear to be due to a disintegration and fusion of proliferating

cells on the superficial layers of the valve leaflets, generally at their most exposed portions, or at sites that form a cul-de-sac in which blood eddies or stasis may occur. Together with this fusion of proliferated cells (endothelium, fibroblasts and other cellular constituents), swelling and eosinophilic changes take place. Whether or not constituents from the plasma are deposited within this material, it is as yet impossible to determine. It appears that the verrucous material is extruded from the valve leaflet because of its swelling and because of cicatrization and contraction of the underlying tissues. Another contributing factor leading to the extrusion of the verrucous material may be the accumulation of inflammatory exudate and the proliferation of swollen basophilic endothelial cells at the base of the verrucae. The fresh verrucae are seldom covered by endothelial cells. The healing stages consist of fibroblastic invasion of the verrucous material with, eventually, complete replacement by scar tissue. Typical granulation tissue capillaries may invade the verrucae (Fig. 6).

The verrucae as a whole in this group were quite extensive and fresh. Moreover, their incidence was high. In 9 of the 12 cases these lesions were present at the closure line of the anterior mitral leaflet. In 3 of the 9 cases they were extensive and spread completely around the tip of the cusp on the ventricularis surface. In 3 additional cases verrucae were observed on the chordae tendineae attachments to the leaflets. Thus, verrucae were observed in every case of this group on the anterior mitral cusp or its chordae tendineae insertions. In 9 cases there were verrucae on the posterior mitral leaflet. Some of these were at the extreme tip of the cusp. Most of them, however, were on the closure line. One additional case showed verrucae on the chordae tendineae insertions (Fig. 5), and another in the posterior mitral pocket. In 9 cases verrucae were present on the closure line of the aortic cusps, and an additional case showed verrucae in the aortic pocket. In 4 cases verrucae were present on the tricuspid valve. An additional 4 cases, however, showed verrucae in the tricuspid pocket. In 2 cases these were seen on the chordae tendineae insertions. Including the pocket and the chordae tendineae insertions, verrucae were present on some part of the tricuspid leaflet in 8 of the 12 cases in this group. Only one pulmonic valve showed verrucae on the closure line. In 2 additional cases, however, verrucae were present in the pulmonic pocket. It is of considerable interest to

note that when the inflammatory lesion did not extend beyond the ring or the base of the pulmonic valve, verrucae tended to occur either in the pocket or in the subvalvular angle. Thus, there appeared to be a tendency for verrucae to localize at a level corresponding to the distal extension of the inflammatory process within the leaflet. This point will be discussed more fully.

As mentioned above, verrucae were noted once in the aortic pocket, once in the posterior mitral, four times in the tricuspid and twice in the pulmonic. Apart from these lesions, most of the valve pockets in this group showed endocardial reduplications, often with mild inflammatory cell infiltrations. These were most notable in the semilunar pockets. At times, the reduplication showed eosinophilic degeneration. This occasionally involved the elastic limiting lamellae, chiefly in the semilunar pockets. Another even more characteristic pocket lesion is polypoid formation (Fig. 8). On cross-section this consists generally of inflamed, finger-like processes, giving the impression of minute polypi. Whether, indeed, these are polypi or whether the spaces between the finger-like processes represent merely dipping down of the endocardium to form vascular channels, it is difficult to determine. The early stages in their formation apparently consist of the extrusion of tiny endocardial hillocks into the valve pocket, *i.e.*, toward the cardiac lumen. These hillocks become elongated and their bases may undergo eosinophilic swelling and fusion. These polypoid lesions were noted in the aortic pocket four times, in the tricuspid once, and in the pulmonic three times.

The incidence of verrucae on the chordae tendineae insertions has already been mentioned. In addition, the chordae tendineae of the anterior mitral cusp showed inflamed reduplications in half the cases. Three of these were vascularized. Four of the cases showed vascularized inflamed reduplications around the insertions of the chordae tendineae in the posterior mitral cusp (Fig. 6). Only 1 case showed reduplications on the tricuspid valve chordae tendineae. Not infrequently the chordae tendineae insertions were agglutinated to one another through the intermediary of verrucous material. Cross-sections of these chordae tendineae often showed swollen basophilic cells scattered between the collagenous bundles. These cells were similar to those described in the inflamed fibrosa layer of the valve.

Although gross vascularization of the valves was inconspicuous in

this group, its incidence microscopically was extraordinarily high. Thus, in the single sections which generally represented each cusp studied, 11 cases of this group of 12 showed blood vessels (generally capillaries) in the anterior mitral cusp, 12 in the posterior mitral, 9 in the aortic, 8 in the tricuspid and 6 in the pulmonic. Moreover, in almost every section blood vessels were present in the valve ring. Thus, if the ring is considered, as it should be, the proximal portion of each cusp, it may be said that almost invariably every cusp of the heart showed blood vessels in this group.

Considered as a whole, the mitral and aortic valves generally showed the widest involvement. However, the leaflets of the tricuspid valve were quite frequently more intensely inflamed than were those of the other valves. In this respect, there was a similarity to the very flagrant involvement of the tricuspid ring. Furthermore, the inflammation seemed to be most severe toward the root of the valve, and edematous widening and hypercapillarization of the auricularis layer were occasionally present. The pulmonic valve generally showed milder lesions. Both the exudative phenomena as well as capillarization were subdued. The most extensive lesions were found in the spongiosa layer.

Summarizing the conspicuous features of the valvular lesions as a whole in Group I, the following points should be noted: The ring lesions \* were extensive, consisting of pronounced capillarization and infiltration with inflammatory cells, sometimes with edema. Blood vessels of the muscular type were infrequent. Aschoff bodies were present in about 10 per cent of the rings. There was little scarring. Practically all the rings and subaortic angles showed lesions. In the latter site, reduplications, when present, were generally not multiple. Approximately half the cases showed involvement of the intervalvular fibrosa.

The lesions in the remainder of the valves generally consisted of intense inflammation, edema and hypercapillarization which involved all portions of the leaflets about equally. There was considerable involvement of the spongiosa and fibrosa layers. Aschoff bodies and eosinophilic swelling of collagen were present in these layers in some cases. The tips of the valves were seldom scarred. The incidence of verrucae was high. These lesions were extensive, fresh, and

\* The descriptions of the ring lesions given in the summary of each group are abstracted from the detailed report by Gross and Friedberg.<sup>48</sup>

showed little organization. Lesions in the pocket and chordae tendineae were frequent. Capillarization of valve leaflets was almost universal.

## GROSS APPEARANCE OF RHEUMATIC VALVES IN GROUP II

### *(7 Active Cases Where One Preceding Attack Occurred Within 1 Year of the Fatal Outcome)*

The most constant gross alteration in this group was a thickening of the valve cusps. Compared with Group I, the thickening was somewhat greater. In no case was the mitral, aortic or tricuspid valve of normal slenderness and translucency. The pulmonic valves, which in Group I were generally normal, revealed in the cases of this group a juicy, succulent consistence with occasional thickening and opacity. Gross vascularization was observed in every case and occurred chiefly in the mitral valve.

Ridge formation at the closure line of the mitral valve, as well as the presence of an overhanging shelf, was quite frequent and much more advanced than in Group I. Corrugation of the auricular surface occurred to the same degree as in the first group.

The aortic valve showed alterations similar to those in the first group, but these were much farther advanced. The thickening was greater and verrucae were present in all cases. The semilunar folds of the aortic cusps were invariably elevated toward the free margins or were completely obliterated (Fig. 10). Rolling and inversion of the free margin and notching at its center were present in 4 of the 7 cases. The notching was due to inversion of the nodulus Arantii into the sinus pocket. Occasionally the nodulus was greatly hypertrophied, forming a knob near the middle of the free border. In 2 cases there was adhesion of the commissures.

Fresh, healing or healed verrucae were invariably present in this group. The location of the verrucae was the same as in Group I except that they were often superimposed upon the ridge on the mitral valve. Furthermore, a double row of verrucae was present on a few valves, one representing healed and one fresh lesions. In addition to the thickening of its cusps, the tricuspid valve invariably revealed verrucae, isolated or in a row.

Abnormalities in the pockets of one or more valves were present in every case. In 4 of the 7 cases there were fresh or healing verrucae in one or more of the valve pockets. These appeared either as tiny,

pinhead-sized yellowish deposits, or as small, yellowish smooth mounds. Occasionally there were other irregularities forming tiny nodular ridges or folds which distorted the normal sharpness of the pocket angle. The alterations of the chordae tendineae were similar to those in the previous group, but the changes appeared more frequently and were more advanced.

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVES IN GROUP II

As noted above, the cusps in this group were somewhat more thickened than in Group I. The thickening was due to the following factors: The proximal layers of the valves generally showed one or more reduplications (Figs. 7, 8 and 9). These were frequently fibro-elastic and occasionally fused with the underlying, generally widened spongiosa. These layers contained numerous inflammatory cells as well as muscular blood vessels, many of which showed typical intimal musculo-elastic hyperplastic changes.\* Indeed, the high incidence of the latter is one of the most conspicuous features of this group. The inflammatory involvement of the valve leaflets showed definite contiguity with the increased ring lesions present in this group. The spongiosa participated notably in these changes. In all the cusps this layer was generally considerably widened with inflammatory exudate and profusely vascularized. The exudative phenomena were even more prominent in this group than in the previous one. As in the latter, the inflammatory cells were chiefly lymphocytes. Occasionally the polymorphonuclear leukocytes predominated. Fibroblasts, plasma cells and macrophages also occurred.

In a previous report it was shown that one of the characteristic features of the ring lesions in Group II is the formation of multiple vascularized elastified reduplications at the subvalvular angles (Figs. 8 and 9). A somewhat similar process takes place in the auricularis layer over the ring region of the auriculoventricular valves (Fig. 7). Not infrequently a prolongation of these multiple vascularized elastified reduplications produces considerable thickening of the cusps.

Another notable feature in this group was the definite involvement of the fibrosa layer. This was generally vascularized or capillarized along the zone that is contiguous with the spongiosa layer.

\* For a detailed description of these vascular lesions see Gross, Kugel and Epstein.<sup>41</sup>



The fibrosa sometimes showed edema, elastica condensation and disruption. Inflammatory cells were numerous. Not infrequently one could trace the vascularization and inflammation of the fibrosa layer along the intervalvular fibrosa. This in turn showed a contiguity process from the aortic ring. In two instances the fibrosa lesion in the mitral leaflet showed such distinct whorling and inflammatory infiltration as to resemble a syphilitic contiguity process from the root of the aorta.

Aschoff bodies were present either in the fibrosa or spongiosa layer in several of the cases. These occurred with approximately the same frequency in all four valves. In addition, the annulus, particularly of the semilunar cusps, occasionally showed eosinophilic swelling of the collagen. Intercryptic cells resembling those seen in Aschoff bodies were also noted in the fibrosa layer, chiefly of the semilunar cusps.

Delicate reduplications of the distal layers of the valves (ventricularis layer of the auriculoventricular cusps and arterialis layer of the semilunar cusps) were frequently present. These reduplications were often inflamed and, in about one-third of the cases, showed vascularization.

A distinct difference from the previous group is the fact that the tips of the cusps almost invariably showed considerable fibrosis and some elastification. Thus, the normally gelatinous tip, which can be considered as forming an extension of the spongiosa layer, was almost always converted into a dense, fibrotic or fibro-elastic, often vascularized, inflamed structure. When the tip becomes collagenous (and, to a lesser degree, elastified), it fuses with a similarly altered auricularis layer to form a thickened ridge. Elastic lamellae from the auricularis and spongiosa layers, together with smooth muscle bundles, may be seen curving beneath this thickened ridge. The tips of the pulmonic cusps, however, rarely showed this collagenous transformation.

The incidence of verrucae still possessing a hyaline structure was highest in this group. Furthermore, the verrucae were generally quite broad and often extended from the closure line around the tip of the leaflet to the ventricularis surface on the auriculoventricular cusps. Verrucae were present on the anterior mitral leaflet in 6 cases. In 2 cases verrucae were noted on the chordae tendineae attachments to this leaflet. Every case showed verrucae on the pos-

terior mitral cusp, two in the posterior mitral pocket, and two on the chordae tendineae insertions. Verrucae were present on all the aortic valves. These lesions were also generally broad and extended around the tip of the cusp, sometimes reaching the arterialis layer. In 1 case verrucae were noted in the aortic pocket. Verrucae were present on the tricuspid leaflets, pockets or chordae tendineae insertions in every case. In only 3 of these were they situated on the closure line or at the tip; in 3 they were present in the pocket, and in 1 on the chordae tendineae insertions. In 1 case verrucae were present on the closure line of the pulmonic valve, and in another they were situated on the auricularis surface at about the middle of the septal leaflet. This case also showed pocket verrucae. In a 3rd case verrucae were present in the pulmonic pocket only.

As mentioned above, verrucae were noted in the aortic pocket once, in the posterior mitral pocket twice, in the tricuspid pocket three times and in the pulmonic pocket twice. Apart from these, other lesions were frequently present at this site. Thus, the great majority of cases showed a knob-like, elastified endocardial reduplication. This generally consisted of a whorled collagenous mass permeated by numerous transverse, discontinuous elastic fibers. The superficial layers of the knob were sometimes intensely elastified and were often the seat of mild or severe inflammation. Occasionally the reduplications were vascularized. An important feature was the tendency for these reduplications to involve the arterialis layer of the semilunar cusps where they shared in the thickening of the valve. In addition, polypoid formations were present in the aortic pocket in 2 cases (Fig. 8), and in the tricuspid in 1 case. The latter sometimes showed agglutination of the chordae tendineae insertions.

The chordae tendineae insertions of the anterior mitral cusp presented considerable agglutinations, with absorption into the valve tip in many instances. They also frequently possessed collagenous, sometimes inflamed vascularized reduplications. These lesions were perhaps more conspicuous in the posterior mitral cusp, where they were occasionally multiple. In one instance an Aschoff body was seen in these reduplications. Chordae tendineae absorption was not as frequently noted in the tip of the tricuspid valve. The reduplications were more delicate and less frequent. However, inflamed vascularized reduplications were noted in two instances.

As stated above, gross vascularization of the valves was frequently

observed in this group. Microscopically, vascularization or capillarization of all the valves was almost invariable. In contrast to Group I, intimal musculo-elastic hyperplastic vascular lesions were conspicuous. Inasmuch as Group I represents a clinical course whose average duration was 6 weeks, it would appear that this lesion generally requires more than 6 weeks for its development. Other observations suggest that the intimal musculo-elastic hyperplastic lesions undergo metamorphosis into intimal fibro-elastification with medial hypertrophy in less than 1 year.

Considered as a whole, the intensity and extent of the inflammatory involvement of the various valves in Group II was similar to that noted in Group I. Perhaps the most extensive involvement occurred in the aortic valve, and in this the contiguity process from the flagrant aortic ring lesion was noticeable. The thickening of the aortic cusps was often largely determined by a prolongation of thick subaortic reduplications on the ventricularis aspect of the valve and, to a lesser extent, by prolongation of aortic pocket reduplications on the arterialis side. The tricuspid lesions were severe, particularly at the root where they merged with similarly severe ring lesions. The pulmonic lesions were the least severe.

Summarizing the conspicuous features of the valvular lesions as a whole in Group II, the following points should be noted: The ring lesions were most extensive and consisted of considerable infiltration and vascularization. The blood vessels were frequently of the intimal musculo-elastic hyperplastic type. The inflammation spread in all directions. The incidence of Aschoff bodies was approximately the same as in Group I. There was considerable scarring. Practically all rings showed involvement. The highly characteristic, multiple subaortic vascularized reduplications occurred in practically every case. The intervalvular fibrosa was invariably involved.

The remainder of the valve leaflets generally showed extreme inflammation and vascularization. Many of the vessels were of the intimal musculo-elastic hyperplastic type. The valves were thicker than in Group I. This was largely due to the more conspicuous reduplications of the proximal layers which were continuous with reduplications at the valvular angles. An important contributing factor to the valve thickening was the widened, inflamed and vascularized spongiosa layer. Involvement of the fibrosa was pronounced. Aschoff bodies were present in some cases. The tips of the

valves were frequently scarred. The incidence of verrucae was higher in this group than in any other of this series. These lesions were extensive and broad; some showed beginning organization. Lesions in the pockets and on the chordae tendineae insertions were similar to Group I. Vascularization of the leaflets was practically universal.

### GROSS APPEARANCE OF RHEUMATIC VALVES IN GROUP III

#### *(11 Active Cases Where One Previous Attack Occurred at Least 2 Years Previous to the Fatal Outcome)*

In this group moderate or great thickening of the valve cusps was universally present in the mitral, aortic and tricuspid valves and in the majority of the pulmonic valves. Compared to the preceding groups this thickening was not only more frequent but definitely greater. Furthermore, it was less uniform, the change tending to become intensely exaggerated in the distal third of the valve cusps, particularly from the closure line to the free margin. In the latter locations, especially in the mitral valve, the cusps were enlarged to form either an exaggerated ridge or plaque. Gross vascularization was frequent.

The surface of the auriculoventricular valves showed greater deformity than in the other groups. These changes were due to rugosities, puckerings and, in 2 cases, to lime deposited diffusely throughout the mitral valve. The cusps of the auriculoventricular valves, particularly the anterior mitral, showed a definite tendency to elongation. This elongation seemed to be due essentially to the formation of new material at the free margins of the cusp, supplemented by absorption of thickened, fused chordae tendineae. The formation of a well marked shelf (overhanging the chordae tendineae of the first order) was quite frequent in these cases. The result of the chordae tendineae absorption was to make them appear definitely shortened, thus bringing the papillary muscles much closer to the margins of the cusps. The characteristic auriculoventricular valve in this group showed greatly thickened cusps with irregular surface, shelf formation and elongation with incorporation of thickened shortened chordae. Chordae tendineae of the second and third order also showed thickening of their insertions. There was stenosis of the mitral valve in 2 cases and of the tricuspid valve in 1.

In addition to thickening, the aortic valves showed notching and considerable shortening. This was due to rolling and inversion of the free margin of the cusps toward the sinus pocket (entropion). Entropion of various degrees was found in approximately half of the cases. In the majority of cases the semilunar folds either approximated the free margin or were completely invisible. Adhesion of the commissures occurred in one-third of the cases. These generally showed the fused margins separated by a delicate slit, characteristic of the rheumatic commissural lesion.

Verrucae in various stages of healing were less frequent than in either of the preceding groups, occurring in about half the cases on the auriculoventricular valves, in 3 of the 8 cases on the aortic valves, and on one pulmonic cusp. There was less tendency for the verrucae to extend onto the chordae tendineae. In the aortic valves, in addition to the previous sites mentioned, the verrucae showed a tendency to extend from one cusp to another across the commissures (Fig. 10). Double rows of verrucae were encountered somewhat more frequently than in the previous group.

Abnormalities in valve pockets were present in all cases. In 1 (tricuspid) there were yellowish masses suggestive of healed verrucae. Whitish nodules, ridges and folds were frequently present. The lining endocardium was often whitened and thickened. In 1 case there was lime in the pocket of the posterior mitral cusp. Occasionally there appeared to be healed agglutinations in the pocket of the auriculoventricular valves.

### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVES IN GROUP III

This group presented qualitative as well as quantitative differences from those previously described. As mentioned in the gross description, not infrequently most of the inflammatory processes apparently occurred with predilection toward the distal part of the cusp, generally within an area relatively confined to the closure line and tip of the valve. The inflammatory process appeared on the whole to be somewhat subdued in comparison with the findings in the first two groups. Reduplications of the auricularis layer of the mitral valve, although still frequently seen and sometimes multiple, were generally notable only at the distal end of the valve. They occurred in only one-third of the cases in the tricuspid valve. Multiple vascularized ventricularis reduplications were almost invariably

present on the aortic cusps but occurred only once in the pulmonic. They consisted of fibro-elastic strata with a tendency toward elastic-collagenous transformation. These reduplications of the proximal layers of the valves frequently fused with the spongiosa layer which was almost invariably involved. As in the previous groups, the spongiosa layer was generally considerably widened, vascularized and elastified and contained inflammatory cells, chiefly lymphocytes. Many of the vessels showed distinct hypertrophy of the media. A few cases showed hypercapillarization. Intimal musculo-elastic hyperplastic lesions were scarce.

The spongiosa layer was occasionally compressed toward the basal portion of the valves and contained distorted capillaries. While the fibrosa layer almost invariably showed involvement with capillaries or muscular vessels within the zone adjacent to the spongiosa, the inflammatory phenomena were on the whole milder than those found in the two previous groups. Elastica changes such as condensations and disruptions were quite frequent. Aschoff bodies and eosinophilic changes were rarely present. One case showed intense whorling of the intervalvular fibrosa collagen and considerable vascularization and inflammation. This showed contiguity with the aortic ring as well as with the mitral valve fibrosa.

The distal layers of the valves presented mildly inflamed reduplications in approximately half the cases. They were on the whole delicate and occasionally vascularized. The mildest lesions of the arterialis layer were found in the pulmonic cusps.

With the exception of the pulmonic cusps, the valve tips were almost invariably converted into collagenous ridges or plaques. As previously described, this was due to fusion of the proximal layers with the spongiosa layer which had undergone collagenous transformation. In the auriculoventricular valves, elastic lamellae from the fused auricularis and spongiosa layers, together with blood vessels and smooth muscle bundles, frequently curved underneath these elastic-collagenous thickenings. The tips of the anterior and posterior mitral leaflets were invariably vascularized. In some instances they were hypercapillarized.

In the gross description it was mentioned that the auriculoventricular valves, particularly the mitral, often showed considerable elongation. This was due to a fusion of the auricularis and spongiosa layers at the tip of the valve with excessive formation of elastic-

collagenous tissue. The collagenous tissue envelops or absorbs the enlarged and fused chordae tendineae insertions, in this manner prolonging the extent of the leaflets.

The tip of the aortic cusps was frequently converted into a collagenous, thickened and rounded edge which represents fusion of the spongiosa and ventricularis layers. On cross-section this formed a knob which occupied almost the entire width of the valve tip and compressed the arterialis layer. The knob itself consisted of radiating fan shaped collagenous bundles whose focal point was situated just below the tip of the valve on its arterial aspect. This focal point not infrequently showed wide, delicate walled vascular channels, probably veins.

In the tricuspid valve ridge formations were inconspicuous, even though the tip was almost invariably collagenous. Knob formation was seen only once in the pulmonic cusp tips.

The verrucae still showing eosinophilic material presented on the whole considerably less reaction at the base than those previously described. Furthermore, their incidence was lower in this group. Thus, the anterior mitral leaflet showed verrucae in 5 cases; in 4 of these they were at the closure line and showed some organization. In 1 additional case they were present on the chordae tendineae insertions. In 5 cases verrucae were noted on the closure line of the posterior mitral leaflet. In most instances these were undergoing organization. An additional case showed verrucae in the valve pocket and one on the chordae tendineae insertions. Four cases showed verrucae on the aortic valve. These were situated on the closure line and presented various stages of organization. In 2 of these cases fresh verrucae were also present in the aortic pocket. Flat organizing verrucae were present on the closure line of the tricuspid valve in 3 cases. In 2 additional cases there were verrucae in the tricuspid pocket, and in 2 others on the chordae tendineae insertions. Thus, the tricuspid valve (leaflets, pockets or chordae tendineae insertions) showed verrucae more frequently than any other valve in this group. In 2 cases verrucae were present on the closure line of the pulmonic cusps and in 2 additional cases in the pulmonic pockets.

As mentioned above, verrucae were found in the valve pockets in this group with the following frequency: in the aortic, twice; posterior mitral, once; tricuspid, twice; and pulmonic, twice. In addi-

tion 5 cases showed polypoid formations in the aortic pocket, 1 in the posterior mitral and 2 in the pulmonic. Practically every pocket showed an elastified reduplication. In some instances these reduplications formed elastified knobs such as described in Group II. In several cases the pocket reduplications were vascularized and mildly inflamed.

The incidence of verrucae on the chordae tendineae insertions has already been mentioned. Reduplications around these insertions were not infrequently seen, particularly in the mitral valve. The reduplications were occasionally vascularized or showed eosinophilic degeneration. In the anterior mitral leaflet, as noted above, the chordae tendineae were occasionally absorbed into the excessively collagenized tip. This absorption was somewhat less frequent in the posterior mitral leaflet and still less in the tricuspid. A point of interest is the fact that in this group, as well as in those subsequently to be described, the spongiosa layer of the valve leaflet opposite the chordae tendineae insertions was not infrequently widened into triangular areas containing many blood vessels.

Vascularization of the valves was invariably present in the anterior and posterior mitral leaflets, as well as in the tricuspid valve. It occurred somewhat less frequently (8 out of the 11 cases) in the aortic valve and only twice in the pulmonic. However, in this group as in those previously described, the rings showed vascularization almost invariably.

This group is the first of the series in which the clinical phenomena were of a more protracted type and the inflammatory processes, therefore, somewhat more indolent. Under such circumstances, as will be more clearly seen in the groups subsequently to be described, contiguity lesions from the ring were not as obvious as in Groups I and II. With a diminution in the intensity of the ring lesions, the inflammatory process either remained confined to the base of the valve or involved chiefly the distal third of the valve, leaving the intervening portions relatively less affected. Thus, while the inflammatory lesion in the tricuspid valve still showed contiguity from the ring and was at times quite intense, it was generally confined to the basal portion. In the pulmonic cusp the lesions were least pronounced and even more frequently confined to the ring. On the other hand, in the mitral and aortic valves the most notable lesions



were at their distal extremities. The significance of these findings will be discussed subsequently.

Summarizing the conspicuous features of the valvular lesions as a whole in Group III, the following points should be noted: The ring lesions were somewhat milder than those in the preceding groups. The vascular lesions consisted of capillaries and muscular vessels in about equal proportions. In some cases vascularization was by means of capillaries only. The incidence of Aschoff bodies was lower than in the previous groups. Intimal musculo-elastic hyperplastic lesions were infrequent. Practically all rings showed involvement. The subaortic angle invariably presented reduplications. In most instances these were multiple vascularized but less conspicuous than in Group II. Group III possessed the highest incidence of subpulmonic lesions, *i.e.*, in approximately half the cases. The intervalvular fibrosa was invariably involved.

The remainder of the valve leaflets generally showed inflammatory infiltration and vascularization of the type found in the ring. Intimal musculo-elastic hyperplastic lesions were severe. Capillaries were sometimes distorted, due to scarring. Auricularis reduplications at the base of the auriculo-ventricular valves were thinner than in Group II. The thickening of the valve was frequently confined to the tip and produced knob formation in the aortic valve. Chiefly in the mitral valve the fibrotic thickened tip was prolonged over the chordae tendineae insertions. The fibrosa layer was invariably involved, but the lesions were generally milder than in the previous groups. Aschoff bodies were infrequent. The verrucae showed a somewhat less reactive base, and many were organizing. Their incidence was lower than in the first two groups. The incidence of pocket lesions as a whole was also somewhat lower than in the previous groups. However, the incidence of aortic pocket polypi was higher than in any of the six groups in this series. This occurred in 5 of the 11 cases. The chordae tendineae insertions frequently showed considerable thickening and absorption into the valve tips. Vascularization of the leaflets exclusive of the ring was invariable in the mitral and tricuspid valves. It occurred in the aortic valve in 8 cases and in the pulmonic in 2. Several cases in this group showed lime formation at the base of the valve.

## GROSS APPEARANCE OF RHEUMATIC VALVES IN GROUP IV

(13 Active Cases Where Repeated Attacks Took Place, Death Occurring During an Acute Recurrence)

Diffuse thickening of the cusps of approximately the same degree as in Group III was universally present in this group. The presence of a pronounced overhanging shelf was observed on the mitral and tricuspid valves in about two-thirds of the cases. Elongation of the auriculoventricular valves, irregularity of the valve surface, and absorption of thickened chordae were present to about the same degree as in the preceding group. The shortening, thickening and fusion of ham shaped chordae tendineae insertions (Fig. 11) were more advanced than in the preceding group. There were occasional agglutinations in the pockets formed by the chordae attachments. Lime was present only in one mitral valve.

The alterations of the aortic valve (Fig. 10) were quite similar to those in the preceding group, being characterized by thickening and shortening of the cusps, approximation of the semilunar folds to the free margin, or their disappearance, rolling, inversion and notching of the free margin, and commissural agglutination.

Fresh and healing verrucae were present in the great majority of all of the valves. Their occurrence, particularly in the tricuspid and pulmonic valves, was much more frequent than in Group III, being present in almost every case. Double rows were seen in a few instances.

Vascularization, chiefly of the mitral valve, occurred with considerable frequency.

Pocket lesions were present in all cases. The pocket angles were generally widened and irregular. The endocardial lining was white or gray and thickened. Yellowish and whitish smooth nodular elevations were often present. Transverse and radial ridges and folds occasionally occurred. Agglutinated verrucae were found in the pocket of one posterior mitral cusp.

## MICROSCOPIC APPEARANCE OF RHEUMATIC VALVES IN GROUP IV

The microscopic lesions of the valves in this group were, on the whole, somewhat similar to those described in Group III. There were, however, several interesting differences which were undoubtedly a reflection of the clinical course (repeated attacks). It was

previously shown that vascular lesions of the intimal musculo-elastic hyperplastic type probably require more than 6 weeks for their development. Their scarcity in Group III indicates that these lesions undergo fibro-elastic metamorphosis after 2 years. (Other observations suggest that such involution may occur in less than 1 year.) Inasmuch as Group IV represents repeated attacks, one of which might have occurred within 1 year before death (thus simulating Group II), it is not surprising that many of the vascular lesions in Group IV were of the intimal musculo-elastic hyperplastic type. However, these were not nearly as conspicuous or as frequently present as in Group II, capillaries and muscular vessels being the predominant type of vascularization. Another interesting feature was the much more frequent occurrence of the collagenous thickening of the valve tips. This will be discussed in greater detail later.

Reduplications of the proximal layers of the valves were generally present. These were frequently delicate in the proximal two-thirds of the leaflets, particularly in the auriculoventricular valves. In this thinner portion of the valve leaflet, vascularization was frequent and often quite superficial. The spongiosa was widened, vascularized, elastified and in general moderately inflamed, particularly in the anterior and posterior mitral leaflets. In the mitral valve the widened spongiosa layer frequently contained large smooth muscle bundles in apposition to the auricularis layer. In the tricuspid valve the spongiosa showed its widening chiefly in the triangular zones above the chordae tendineae insertions.

The fibrosa layer of the auriculoventricular valves almost invariably showed moderate inflammation with some elastification and elastica distortion. The fibrosa layer of the aortic valve showed little involvement. Most of the cases presented intercryptic swollen cells in the fibrosa of the pulmonic cusps. These cells were generally less abundant in cytoplasm than were those found in the previous groups. In 1 case there were whorling and vascular permeation of the fibrosa collagen in the anterior mitral leaflet. This inflammatory lesion could be traced through the intervalvular fibrosa to the aortic annulus. Aschoff bodies were observed in only one valve. This was in the aortic fibrosa. On the whole it may be said that the fibrosa lesions in this group were the mildest of those thus far described, but they were still present in the majority of instances, except in the aortic cusps.

The ventricularis layer of the auriculoventricular cusps showed delicate collagenous reduplications. In a few instances these were vascularized and presented mild inflammatory changes. Similar changes occurred in the arterialis layer of the semilunar cusps. Inflamed arterialis reduplications occurred more frequently in the aortic than in the pulmonic cusps.

In almost every case each valve tip, except the pulmonic, showed fibrosis. The tips of the anterior and posterior mitral leaflets were thickened, elastified, collagenous and vascularized. As in previous groups, this was due to fusion of the collagenous spongiosa and auricularis layers. Redundant collagen from these fused and thickened valve tips frequently spread for a considerable distance over the chordae tendineae insertions and produced elongation of the cusps. Deviation of the auricularis and spongiosa elastic lamellae, blood vessels and smooth muscle bundles were generally noted beneath the thickened fused mass at the tip. On cross-section the tips of the aortic valves were represented by large collagenous knobs in every case (Fig. 12). Histologically these were similar to those described in Group III. The pulmonic cusps were the least involved and showed knobs only occasionally. The tricuspid valve tips were generally collagenous and sometimes showed extension over the chordae tendineae, but extensive thickening was infrequent.

The verrucae present in this group were generally of a more indolent type than those found in the previous groups. Many of these were flat, on a broad base and with little reaction in the underlying tissue. A number of them showed considerable organization and absorption within the leaflet. In a few cases the verrucae appeared to represent merely an eosinophilic degeneration of the superficial collagenous layer of the valve leaflet, chiefly at the closure line or around the tip of the valve.

The anterior mitral cusp showed fresh verrucae in 6 cases. These were generally situated at the closure line or near the tip. Some were undergoing organization. In 1 of these cases verrucae were also found on the chordae tendineae insertions. In 9 cases the posterior mitral cusp showed verrucae. Most of them were organizing. Verrucous lesions were present in one of the posterior mitral pockets. No verrucae were found on the chordae tendineae insertions of this cusp. In 7 cases verrucae were present on the aortic cusp. These were situated either on the closure line or around the tip. They were

generally broad and showed relatively little reaction. In 1 case verrucae were also present in the aortic pocket. Ten of the cases showed verrucae on the tricuspid valve, an unexpectedly high incidence, and the highest in this group. These were generally broad also, many resembling eosinophilic change. Some were undergoing organization. Three of the cases showed verrucae in the tricuspid pocket and 3 on the chordae tendineae insertions. Thus, in 12 of the 13 cases in this group, fresh verrucae were present on some portion of the tricuspid valve. Chordae tendineae agglutinations in the tricuspid pocket were also sometimes seen. Obviously, therefore, the tricuspid valve lesion, although leading to less fibrosis than the other cusps, maintains activity in an extraordinarily high percentage of cases. In 5 of the cases verrucae were present on the pulmonic cusps. These also were generally flat or organizing. Two cases showed verrucae in the pocket. In 6 of the 13 cases in this group verrucae were present on some portion of the pulmonic valve. This represents the highest incidence of pulmonic valve verrucae of any group and is, undoubtedly, a reflection of the multiple attacks.

As mentioned above, verrucous lesions were found in the valve pockets with the following frequency: once in the posterior mitral, once in the aortic, three times in the tricuspid and twice in the pulmonic. In addition, the aortic pocket contained polypoid lesions in 3 cases, the posterior mitral in 2, and the pulmonic in 1. Most of the cases showed elastified distorted pocket reduplications, some of which formed large knobs. In a few cases vascularized reduplications were present in the pockets.

The incidence of verrucae on the chordae tendineae insertions has already been referred to. As noted, absorption within the mitral leaflet tip was almost invariably present. Multiple vascularized inflamed reduplications were present on some chordae tendineae insertions of the mitral valve. In a number of cases many of the chordae tendineae insertions of the tricuspid valve also showed absorption.

Vascularization was present grossly and microscopically in almost all the cusps except the pulmonic, where it was noted in 6 of the 13 cases in this group. Ring lesions with vascularization were almost invariably present.

Even though the inflammatory lesions on the whole were not as varied in Group IV as in the other groups described, there were

present other manifestations of continued damage which placed this group after the first two in order of activity. On the other hand, because of the somewhat prolonged course and repeated attacks, there were present also evidences of chronicity which approximated the changes present in the groups subsequently to be described. It is, therefore, this combination of fairly active lesions and extensive healing which characterizes this group. The greatest distortion and thickening of the valve leaflets occurred in the posterior mitral cusp. Of interest was the fact that the tricuspid valve was still consistent in showing notable exudative lesions. Inflammatory involvement of the pulmonic valve was not infrequently most pronounced in the middle portion of the cusps. This is of considerable interest as indicating the tendency for lesions in this valve to be arrested before spreading to the tip.

Summarizing the conspicuous features of the valvular lesions as a whole in Group IV, the following points should be noted: The ring lesions consisted only of distorted capillaries caused by the scarring process. The occurrence of inflammatory cells was less frequent than in the previous groups. Aschoff bodies were most infrequent. All the rings were involved. All the subaortic angles showed lesions that were almost invariably of the multiple elastified variety. These, however, were not as great as in the first two groups. The intravalvular fibrosa showed a high incidence of lesions (11 of 13 cases).

The remainder of the valve leaflets generally showed somewhat milder exudative phenomena than in Group III. Vascularization was similar to Group III but, in addition, intimal musculo-elastic hyperplastic vessels were more frequent. Because of more definite scarring, distorted capillaries were frequently seen. In some instances the proximal two-thirds of the valve leaflets were fairly thin and showed superficial vascularization with thick vessels. In others the valve was diffusely thickened. Marked fibrosis and thickening of the tips of the mitral, aortic and tricuspid valves were noted more frequently in this group than in the others thus far described. The most notable thickening was that in the posterior mitral leaflet. This thickening of the tips of the auriculoventricular valves, together with elongation of the leaflets, was due to the same process as previously described. The fibrosa of the auriculoventricular valves almost invariably showed a mild degree of inflammatory involvement.

The aortic fibrosa was generally intact. That of the pulmonic valve showed intercryptic cells with basophilic cytoplasm. Aschoff bodies were found in the fibrosa only once.

The verrucae in this group showed even more indolence than in Group III. A number of them consisted of eosinophilic, swollen and degenerated collagen with little reaction at the base. On the other hand, the incidence of verrucae in this group was higher than in Group III, though somewhat lower than in the first two groups. The incidence of polypoid and verrucous pocket lesions also placed this group third in order of frequency. The incidence of verrucae on the chordae tendineae insertions and the absorption of the latter into the valve tip were somewhat similar to Group III. Vascularization of the valves was extremely frequent, occurring almost invariably in every valve except the pulmonic, where it was noted in 6 of the 13 cases in this group.

#### GROSS APPEARANCE OF RHEUMATIC VALVES IN GROUP V

*(28 Active Cases Where Death Was Caused by Decompensation Without Clinical Evidence of a Final Acute Attack. Some of These Cases Had No Previous History of Rheumatic Fever)*

The cases in this group showed the most advanced alterations of any group. Definite diffuse thickening of the cusps was universally present in the mitral, aortic and tricuspid valves, and there was moderate thickening of the pulmonic valve. The formation of a ridge or thickening in the peripheral portion of the mitral cusps was more frequent than in the preceding group, being present in half the cases. A pronounced overhanging shelf also was present on the mitral valve in half the cases. In 9 cases the valve was diffusely infiltrated with lime which notably distorted the cusps by its projection through the auricular and ventricular surfaces. In a number of instances vertical cracks appeared through the lime at the commissural regions. Buttonhole stenosis of the mitral valve was present in 4 cases.

As in the preceding group, the aortic valves were greatly thickened, shortened, and their edges rolled and inverted. Notching was most frequent. There was a much greater tendency to the deposition of lime in the cusps themselves, in the region of the noduli, and in the commissures. Adhesions of the cusps at the commissural margins was much more frequent than in the preceding group. The

valves in general appeared much more distorted and were rigid, a characteristic not found in the preceding group.

Fresh and organized verrucae were less frequent than in the preceding group, being present in 6 cases on the mitral valve, in 10 on the aortic, in 12 on the tricuspid and once on the pulmonic valve. Double rows of verrucae were observed in several cases. Thickening, fusion, absorption and shortening of the chordae tendineae were more pronounced than in the preceding groups. Generally, only the valvular attachments of the chordae tendineae of the third order were still present. Not infrequently the papillary muscles were almost in contact with the valve margins.

Gross vascularization occurred with considerable frequency and was found chiefly in the mitral, aortic and tricuspid valves.

The pockets were characterized chiefly by a whitening and thickening of the endocardium; there was a tendency for the auriculoventricular valve leaflets to form agglutinations with the ventricular wall and thus obliterate the sharp pocket angle. Further irregularities and obliteration of the auriculoventricular pocket angles were due to the frequent presence of fibrous bands and muscular bridges at this site. The pockets of the aortic cusps and, to a less extent, of the pulmonic cusps, frequently contained nodules, ridges and folds.

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVES IN GROUP V

The auricularis layer of the auriculoventricular valves as well as the ventricularis layer of the aortic valve showed multiple elastified reduplications in approximately half the cases. These were frequently quite thick and contained sparse scatterings of lymphocytes. Fusion of the elastic-collagenous terminations of these layers with the similarly transformed tip of the spongiosa layer produced considerable thickening. The reduplications on the proximal valvular surfaces were all vascularized in the tricuspid valve, and generally in the posterior mitral leaflet and aortic valve. Only a few cases showed vascularization of the auricularis layer in the anterior mitral leaflet. In the pulmonic cusps the ventricularis layer was either intact or showed more delicate reduplications, only a few of which were vascularized. The spongiosa layer of the valves was almost invariably thickened and vascularized, and generally mildly inflamed. The vascularization, particularly in the spongiosa layer, consisted of greatly hypertrophied muscular vessels, sometimes of the intimal



musculo-elastic hyperplastic type. In a few instances the collagenous transformation of the auricularis layer produced compression of the spongiosa layer.

Although 1 case showed definite whorling and vascularization of the fibrosa collagen, this layer was much less frequently involved than in the previous groups. In this respect Group V differed greatly from the preceding groups. Furthermore, in this group lipoid and calcific deposits involving the spongiosa as well as the fibrosa layers occurred considerably more frequently. It is to be noted, however, that the average age period of this group was somewhat older than the preceding. Aschoff bodies were seen only once. These were present in the anterior mitral leaflet. The fibrosa layer of the pulmonic valve generally showed intercryptic cells.

Most of the auriculoventricular valves showed delicate collagenous ventricularis reduplications. These were considerably exaggerated in width at the site of the chordae tendineae insertions, and on microscopic section presented the appearance of conspicuous crescents. In many instances, particularly on the mitral valve, the ventricularis reduplications were vascularized. In several instances the arterialis layer of the aortic valve consisted of widened collagenous extensions of pocket reduplications. These showed moderate inflammation, sometimes vascularization, and extended as far as the tip of the cusp, thus increasing the thickness of the leaflet. In several cases the arterialis reduplications were enormously thick and were associated with entropion of the aortic valve tip. The arterialis and ventricularis layers of the pulmonic valves were either uninvolved or showed delicate reduplications.

The tips of the mitral (Fig. 13), aortic and tricuspid valves were practically all converted into elastic-collagenous masses. These thickened tips were almost invariably vascularized and showed elastic bands and smooth muscle curving under the fused auricularis and spongiosa terminations. Excessive collagen formation with absorption of fused chordae tendineae insertions and elongation of the cusps was frequent in the auriculoventricular valves (Figs. 14 and 15). Some leaflets showed a moderate degree of inflammatory reaction. The tip of the pulmonic valve frequently showed fibrosis but the formation of knob-like thickening was seen in only one instance.

In spite of the fact that in the cases in this group death took place from decompensation without clinical evidence of a final acute at-

tack of rheumatic fever, and that some of these had no previous history of this condition, approximately 20 per cent of all the valve leaflets showed verrucae. This, together with the invariable presence of Aschoff bodies in the myocardium of these cases, as well as the mild inflammatory lesions in the cusps and elsewhere, indicates that decompensation in rheumatic valvular disease is frequently an evidence of activity of the rheumatic process, as contended by Rothschild, Kugel and Gross,<sup>48</sup> and others.

In the majority of instances the verrucae in this group were broad, flat, extremely indolent, showed little or no reaction at the base and presented the appearance of eosinophilic collagen degeneration of the superficial layers of the valve. A number of verrucae showed advanced organization. Completely organized verrucae were represented by ridges of proliferated fibroblasts at various levels on the valve tips. In 5 cases fresh or organized verrucae were situated either on the closure line or at the tip of the anterior mitral leaflet. In 2 of these the lesions resembled eosinophilic collagenous degeneration. An additional case showed a similar process involving the insertions of the chordae tendineae. In 6 cases organizing verrucae were present on the closure line or at the tip of the posterior mitral cusp. In 1 case these were also present on the chordae tendineae insertions. In 6 cases the aortic cusp showed fresh or organizing verrucae on the closure line or tip. Most of these were of the nature of an eosinophilic swelling and degeneration. In 7 cases flat verrucae or eosinophilic collagenous degeneration were present on the closure line of the tricuspid valve. One of these cases also showed verrucae on the chordae tendineae insertions. In 2 cases there were indolent verrucae on the closure line of the pulmonic cusps.

None of the valve pockets in this group showed verrucae. On the other hand, scarring of the underlying annulus and delicate elastified reduplications were present in all the valve pockets. The reduplications were inflamed and vascularized in the posterior mitral pocket in 4 cases and in the tricuspid valve pocket in 6 cases. Occasionally the pocket reduplications were multiple. The pulmonic pocket was the least involved. Besides these lesions, one aortic pocket showed a polypoid structure. Deposition of lipid crystals in the pockets, especially toward the later age periods, was occasionally seen.

As mentioned above, the chordae tendineae insertions of the mitral and tricuspid leaflets showed verrucae in a few cases. In

several instances the reduplications were multiple and vascularized. In many cases the chordae tendineae insertions were absorbed into the elastic-collagenous valve tip and incorporated into the collagenous extension of this structure with resulting elongation of the cusps. Agglutination of the chordae tendineae to the endocardium was frequently noted in the tricuspid pocket.

Gross and microscopic vascularization was almost invariably noted in the mitral and tricuspid valves. Only 17 of the 28 cases in this group showed vascularization of the aortic valve. Vascularization of the ring was present in these and in 4 additional cases. In 13 cases the pulmonic valve showed vascularization. Vascularization of the ring was present in these and in 6 additional cases. Thus far, therefore, in the five groups described, universal vascularization of either valve leaflets or rings was found in the great majority of cases.

Inflammatory phenomena were extremely indolent in this group. However, when activity was present it still appeared to be most noticeable in the tricuspid leaflet. In a number of cases the exudative phenomena seemed to stop at the rings. Although vascularization was not present in the various valve leaflets of a number of cases, the proximal valve layer and the spongiosa layer not infrequently showed considerable thickening with transformation into collagenous ridges. Indeed, some of these cases showed indolent verrucae and one presented Aschoff bodies. This suggests the possibility that a low grade toxic process proceeding from the ring was able to elicit this gradual inflammatory transformation of the valve leaflet, chiefly at its distal portion, but was insufficient to stimulate the formation of blood vessels.

Not infrequently the posterior mitral valve was the one to show the greatest thickening process. This was often confined to the tip of the valve, the proximal part showing little more than superficial vascularization involving only the auricularis layer.

A number of the valve leaflets which were intact in other respects showed elastica condensations and ruptures in the spongiosa layer. In many cases the pulmonic cusps were notable for the complete absence of inflammatory cells. However, the relatively high incidence of vascularization in this valve indicated a previous inflammatory process.

Summarizing the conspicuous features of the valvular lesions as a

whole in Group V, the following points should be noted: The ring lesions showed considerable diminution in their extent, intensity and incidence. The blood vessels were generally thick walled arterioles or arteries, or distorted capillaries. The rings showed scarring and elastica distortion. Cellularity was sparse and Aschoff bodies rare. Subaortic lesions occurred in approximately half the cases. These were generally of the multiple vascularized variety. The incidence of the intervalvular fibrosa lesion was approximately 50 per cent. This was generally mild. In contrast with the previous four groups, universal ring lesions occurred in approximately half the cases. Ring lesions were found in three rings in another 25 per cent of the cases. Every case showed involvement of at least two rings.

The remainder of the valve lesions generally showed extremely mild exudative phenomena. Vascularization consisted of thick walled vessels and of capillaries, often distorted by scar tissue. Intimal musculo-elastic hyperplastic vessels were scarce. Thickening of the valve leaflet as a whole, due to somewhat heavier reduplications, was more frequently seen than in the last group. Thickening and elastification of the valve tips was also more frequent. On the other hand, the incidence of relatively intact leaflets was higher in this group than in the preceding one. As in Group III, the posterior mitral leaflet frequently showed the greatest thickening, and the tricuspid valve the greatest activity. Deformity of the aortic valve was often due to enormously thickened ventricularis and arterialis layers. The fibrosa layer was much less frequently involved than in the preceding groups. On the other hand, lime deposits in this as well as in the spongiosa layer occurred with considerable frequency. Aschoff bodies were found in 1 case. Verrucae occurred in approximately 20 per cent of the cases, the lowest incidence of any of the groups thus far described. These lesions appeared most frequently in the tricuspid valve and, in most instances, consisted of eosinophilic swelling and degeneration of the superficial layers of the leaflet at the tip. Polypoid lesions were found in only 1 case. This was in the aortic pocket. No pocket verrucae were present in this group. The chordae tendineae insertions showed crescentic reduplications in many cases and were often greatly thickened. Even though vascularization of the mitral and tricuspid valves was almost invariable, the incidence of vascularization of the

aortic valve decreased considerably (17 of 28 cases). The pulmonic valve was vascularized in 13 cases.

#### GROSS APPEARANCE OF RHEUMATIC VALVES IN GROUP VI

##### *(26 Inactive Cases of Chronic Valvular Disease of the Typical Rheumatic Variety)*

The changes in this group varied considerably in severity. Some of the cases showed the extreme valvular deformities that have been described in Group V. There were many instances, however, in which one or more of the valves was either entirely normal or showed only a mild or moderate thickening. In selecting the cases for this group there were excluded those that presented the generally large secondary thrombotic verrucous lesions which have been separately described by Gross and Friedberg<sup>52</sup> under the classification of non-bacterial thrombotic endocarditis.

The mitral valve generally showed distinct thickening. Healed verrucae were present in only 4 cases out of the 26. Vascularization was visible in 14 cases. In 9 the terminal portion of the valve was converted into a collagenous mass. Lime was present in 6 cases, and in 2 there was definite stenosis of the valvular surface. In only one-fifth of the cases was there extreme deformity of the valve with elongation and absorption of thickened, shortened chordae tendineae.

Half of the cases showed only a mild or moderate thickening of the aortic valve. Healed verrucae were present in 1 case. In 4 cases there was a shortening of the cusp with notching, rolling and inversion of the margin, as previously described. In about one-fourth of the cases the semilunar folds approximated the free edge, or were obliterated. In 3 cases the cusps were rigid, chiefly because of infiltration of lime. Adhesion of the cusps at their commissures was present in 8 cases.

Most of the tricuspid valves showed only a mild or moderate diffuse thickening. Healed verrucae were present on three of the valves. Occasionally there was a loss of normal scalloping or an early shelf formation. In a few cases there was shortening and thickening of the chordae tendineae. The pulmonic cusps showed a mild degree of thickening in about two-thirds of the cases. In the remaining cases the valve was normal.

Abnormalities in the valvular pockets were present in about two-thirds of the cases. These abnormalities were qualitatively similar

to those in the preceding group, consisting of whitening and thickening of the endocardial lining, the presence of ridges, tiny nodules, folds and muscular and fibrous bridges which distorted the regularity of the pocket angle.

# MICROSCOPIC CHANGES OF RHEUMATIC VALVES IN GROUP VI

Practically all the mitral valves in this group showed collagenous auricularis reduplications which were generally quite flat in the proximal two-thirds of the valve, and possessed superficial vascularization with muscular vessels. In many cases the tip of the valve was converted into a collagenous mass. About half of these thickened tips were vascularized. Practically every case showed reduplications of the aortic valve ventricularis. In one-third of the cases these were multiple, and in one-half the cases they possessed enormously thick vessels with narrowed lumens. The lesions of the tricuspid valve were generally much less striking. The pulmonic valve generally showed either a delicate collagenous reduplication of the ventricularis layer or no discernible lesion.

The only consistent spongiosa lesion found in this group was in the posterior mitral cusp. In half the cases this layer was widened, elastified and vascularized. The widening was particularly noticeable over the chordae tendineae insertions. The blood vessels in the spongiosa layer were generally greatly hypertrophied and often showed intimal narrowing. Sparse scatterings of mast cells and lymphocytes were occasionally noted. In the tricuspid and aortic valves, vascularization of the spongiosa layer was infrequent. In the pulmonic valve it was rare. Chiefly in the mitral valve, the spongiosa sometimes showed considerable compression by the collagenous auricularis.

Apart from degenerative changes (hyaline transformation or lipoid or calcific deposition), the fibrosa layer was practically uninvolved in these cases. Occasionally it was thinned. In 1 case this layer showed whorling of the collagen and vascularization.

In 3 cases the ventricularis layer of the anterior mitral leaflet showed multiple reduplications. In 4 they were vascularized and richly elastic-collagenous. Apart from these, about half the cases showed crescentic reduplications of the chordae tendineae insertions. In the posterior mitral leaflet and in the tricuspid valves the ventricularis layer showed little else than crescentic thickenings of

the chordae tendineae insertions. In half the cases the aortic valve arterialis showed collagenous reduplications. The arterialis of the pulmonic valve was practically intact.

The tips of the mitral valve were invariably converted into elastic-collagenous thickenings. In two-thirds of the cases these thickened tips were vascularized. The tricuspid valve showed considerably less thickening of the tip. On the other hand, two-thirds of the cases showed extreme collagenous thickening of the aortic valve tips. These were less frequently vascularized than the mitral and generally consisted of the fan shaped collagenous knob structure described above. The tips of the pulmonic cusps showed no collagenous thickenings or other changes.

The only lesions in any way resembling verrucae found in this group were eosinophilic swelling and degeneration occurring generally on the closure line and showing practically no reaction of the underlying structure. These lesions were, on the whole, delicate and were found once on the anterior mitral leaflet, once on the posterior mitral, three times on the aortic and once on the tricuspid.

No verrucae or polypoid lesions were found in the valve pockets. On the other hand, all the valve pockets showed elastified reduplications. These were generally quite delicate and, in a few instances, vascularized. No verrucae were found on the chordae tendineae insertions. These frequently showed absorption into the mitral valve tip. In about half the cases the chordae tendineae insertions into the mitral and tricuspid valves showed crescentic reduplications. In 1 case the reduplications were vascularized.

The incidence of vascularization of the valves as a whole showed a conspicuous difference from the previous groups. Thus, while the anterior mitral leaflet was almost invariably vascularized, capillaries or muscular vessels were present in 20 of the 26 cases in the posterior mitral leaflet, in 12 cases in the aortic, in 8 cases in the tricuspid and only in 4 cases in the pulmonic. In 9 additional cases vascularization was present in the aortic ring, in 9 in the tricuspid ring and in 11 in the pulmonic ring.

Considered as a whole, this group showed a considerably lower incidence and severity of valvular deformities. Reduplications of the proximal valve layers were either delicate or not present. The mitral valve showed the most advanced lesions. Vascularization, when present, was frequently quite superficial on the proximal two-thirds

of the leaflets. Angle lesions, both over the auriculoventricular and semilunar rings were inconspicuous. Although the tricuspid valve was practically free from exudative phenomena, capillaries were still conspicuous. The pulmonic valve was notable for the frequency with which it was intact and for the slight extent of the deformity when it was involved.

Summarizing the conspicuous features of the valvular lesions as a whole in Group VI, the following points should be noted: The ring lesions showed practically no exudative phenomena. Aschoff bodies were not present. Inflammatory cells were extraordinarily sparse, scarring was appreciable and vascularization consisted of capillaries, hyaline arterioles or hypertrophied vessels. Subaortic lesions occurred in approximately half the cases. Half of these were multiple elastified reduplications and only half of these again were vascularized. Only 1 case of the 26 in this group showed a subpulmonic lesion. Intervalvular fibrosa lesions were found in only 5 of the cases. These were extremely mild. Only 6 cases showed universal ring involvement and in another 7, three rings were involved. Every case showed involvement of at least one ring.

The remainder of the valve leaflets was equally free of exudative phenomena. Vascularization was by means of capillaries and extremely thick walled vessels with greatly narrowed lumens. These were usually found in the superficial layers of the valve. Many valves were completely intact or showed some elastification of the fibrosa layer. Thickening of the leaflets occurred chiefly in the mitral valve and usually affected the tip in a manner similar to that described for the previous two groups. The only fibrosa lesion present was deposition of lime, which occurred in several cases. No Aschoff bodies were present. The only changes approximating verrucae were found in but few valves. These consisted of eosinophilic degeneration of a completely bland type. The pockets generally showed delicate reduplications and were free from verrucae or polypoid lesions. When involved, the chordae tendineae insertions showed reduplications generally of the crescentic variety. The anterior mitral leaflet was the only one which was almost universally vascularized. Blood vessels were found in 20 of the 26 cases in the posterior mitral leaflet, in 12 in the aortic, and in 4 in the pulmonic.



## DISCUSSION

In a previous report <sup>46</sup> it was shown that the rings are likely to be the first parts of the valves involved in a rheumatic affection. Briefly considered, the observations which support this view are as follows: (1) the valve rings almost invariably show inflammatory changes even when the remainder of the valve leaflets is relatively free from disease; (2) the lesions in the rings are generally more flagrant than those in the rest of the leaflets; and (3) particularly in the active cases, a definite contiguity inflammatory process can usually be seen extending from the valve ring into the body of the leaflet.

In the same report a discussion was given of the mechanisms concerned with the localization of the inflammatory process in the valve rings and with the spread of these lesions to and from the several rings. It appears that three mechanisms may play a rôle in this connection: (1) the simultaneous involvement of all the valve rings by the spread of infection from the adjacent myocardium; (2) involvement of the aortic and pulmonic rings by spread from the roots of the great vessels; and (3) involvement of the mitral ring from the inflamed left auricle with contiguity spread to the other rings, chiefly by way of the annulus extensions, but also through the pericardial wedges. It was shown that blood vessels do not exist in most normal rings and that they are probably not concerned with the initial localization of the rheumatic lesions in these sites.

The observations incorporated in the present report afford an excellent opportunity to study the pathogenesis of the valvular lesions and their life cycles. Assuming that the initial valvular lesion is in the ring, it appears that spread of the infection takes place by a contiguity process chiefly through the spongiosa layer, as well as the proximal layers of the valves (auricularis layer of the auriculoventricular valves and ventricularis layer of the semilunar valves). The spongiosa layer undergoes considerable widening, with edema and inflammatory infiltration. The proximal layers of the auriculoventricular and semilunar valves are prone to the formation of reduplications whose structure is somewhat similar to that previously described in the left auricle (Gross <sup>43</sup>). Thus, the generalized valvular thickening which occurs during an initial attack or an acute exacerbation is due partly to extensive exudation and partly to the formation of these reduplications of the proximal layer of the valves. It

appears further that verrucae result from certain exudative and degenerative processes which occur on the proximal surface of the inflamed valves, chiefly at the closure line. These processes are frequently associated with proliferation of the local fixed cells. These often arrange themselves at right angles to the surface and form the so-called palisades. This, therefore, is not a primary lesion as believed by Leary,<sup>34</sup> but occurs subsequent to the valvulitis. The secondary rôle in the thickening process played by the formation of verrucae will be considered separately.

In the more chronic stages, deformity of the auriculoventricular valves is produced by thickening of these reduplications, formation of multiple reduplications, elastic-collagenous transformation of the tips of the leaflets and, in some cases, lipoid and calcific deposition in the annulus around the pocket, the fibrosa layer and the thickened tip. If the lesion is of long duration there may be added to these, thick reduplications on the ventricularis layer. Together with these processes other changes generally occur. Thus, fibrosis of the auriculoventricular valve tips is frequently associated with excessive collagen formation, part of which results from organization of verrucae. In the auriculoventricular valves this collagen spreads over and fuses with the chordae tendineae insertions, which are thickened by fibro-elastic reduplications, with resulting elongation of the valve leaflet. These elongated leaflets, with their incorporated chordae tendineae, are molded together by redundant cicatrizing connective tissue as a result of which there is eventually created a rigid, flattened or rounded, funnel shaped structure — the typical valvular stenosis. A similar process, in the absence of redundant connective tissue, leads to a less marked stenosis without lengthening of the valve leaflets — indeed, at times with shortening due to cicatrization. Thus, the formation of auricularis, ventricularis and chordae tendineae reduplications, the profuse collagenization of the valve tips and the fusion of the thickened chordae tendineae insertions with the thickened valve tips are responsible for the characteristic deformities of the auriculoventricular valves in chronic valvular disease. To these may be added the secondary lipoid and calcific changes and, in some cases, secondary thrombotic deposits on the closure line with organization (Gross and Friedberg<sup>52</sup>).

Deformity of the semilunar cusps occurring in the chronic cases is due to a somewhat similar process, modified, however, by the to-

pography of the commissures and by the absence of chordae tendineae. The close approximation of the inflamed semilunar folds between two cusps frequently leads to their agglutination. This produces the characteristic rheumatic commissural lesion in which a delicate groove persists between the agglutinated cusps. Eventually, thickening of the cusps and commissures leads to stenosis. The redundant collagen formation at the tip of the ventricularis layer, which is partly due to organization of verrucae, causes the semilunar cusps to fold over or become fused with a thickened arterialis layer (entropion). The latter, as has been shown, rarely undergoes the excessive collagen transformation seen in the ventricularis layer. In some instances the tips of the cusps fold over toward the lumen of the heart, producing ectropion. It is seen, therefore, that in contrast to the auriculoventricular valves the semilunar cusps are shortened by the infolding (or outfolding) of the tip. As a consequence, the semilunar folds on these cusps approximate the newly formed free edge and may eventually disappear completely. Furthermore, the infolding of the nodulus Arantii produces a distinct notching at the center of the free edge of each cusp.

It is clear from the above that the presence of chordae tendineae permits the redundant collagenous tissue to fuse with them and produce elongation of the auriculoventricular valves. On the other hand, their absence on the semilunar cusps causes the same inflammatory process to produce shortening and generally entropion of the semilunar valves with notching and approximation of the semilunar folds to the free edge or their disappearance. Obviously, the degree to which deformity will take place depends on the acuity of the inflammatory processes, on their repetition, on the valve affected, as well as on the type of reaction elicited in the given case. The latter plays a prominent rôle in determining the extent of the secondary lipoid and calcific changes which occur. As has been emphasized by Libman,<sup>53</sup> there are undoubtedly definite individual differences in the propensity for the deposition of calcific material in blood vessels, pericardial exudate, valve rings and valve leaflets.

The deposit of lime in the cusps in turn leads to characteristic gross and histological changes. It has been shown above that the fibrosa layer of the cusps becomes hyaline and relatively acellular with advancing age periods and that, simultaneously with these changes, lime deposition takes place. Apparently the same process

occurs in the definitely collagenous, hyaline and relatively acellular structure found in the scarred ring annulus, fibrosa layer at the base of the valve, and thickened valve tips. As has been shown for certain blood vessel lesions, rheumatic fever may greatly hasten a normally occurring degenerative process. It has been indicated before that these changes are apt to occur with predilection at the commissural regions. Secondary to the lime deposition, granulation tissue capillaries and inflammatory cells may surround the foreign material and lead to agglutination of the cusps. At times the calcific material in the commissures, particularly of the mitral valve, shows characteristic vertical cracks. These sites may be covered with thrombotic material and become infected with bacteria, thus producing a bacterial endocarditis. Metaplasia with bone and bone marrow formation may occur in the areas of lime deposition.

It was previously shown that the lesions in Group I (cases with death during a first attack) are characterized by the acuity and extent of the exudative phenomena and by the fact that the vascularization of the valves is mainly of the capillary type. Reasons were given which indicate that 6 weeks are generally insufficient for the production of muscular wall vessels by the rheumatic process. In the Group II cases the patients had suffered one previous attack within 1 year of the fatal outcome. In these the exudative phenomena were even more distinct. In addition, characteristic reduplications were present at the valve angles and on their surfaces, and the vessels present in the inflamed valves were frequently of the intimal musculo-elastic hyperplastic type. It appears that this type of vessel lesion, therefore, apparently generally requires more than 6 weeks for its production. In Groups III, IV and V the active exudative phenomena became increasingly indolent and were absent in Group VI. The vasculature in the valves of these more chronic groups consisted of capillaries, often distorted by scar tissue, and of vessels with muscular walls, and sometimes intimal fibrosis (particularly in Groups V and VI).

Of great interest is a consideration of the verrucous lesions found in these cases. Their incidence was unexpectedly high and apparently closely paralleled the clinical course of the disease. Thus, verrucae were almost invariably present on the mitral, aortic and tricuspid valves (including the pockets and chordae tendineae insertions) in Group II, and occurred with high frequency in these

valves in Group I. Group IV was the next in order of frequency as regards the incidence of these lesions. This was not unexpected in view of the fact that this group represents cases with repeated attacks. Furthermore, inasmuch as one of these rheumatic bouts occurred within 1 year of the fatal outcome in a number of cases (thus simulating Group II), the incidence of intimal musculo-elastic hyperplastic vascular lesions was also somewhat high in this group. Verrucae occurred in approximately 50 per cent of the valves in Group III. Their incidence fell considerably in Group V, occurring in approximately 20 per cent of the valves. In Group VI they were rare and consisted entirely of degenerated eosinophilic material resulting from collagen necrosis. The relation of these lesions to non-bacterial thrombotic endocarditis (Gross and Friedberg<sup>52</sup>) will be subsequently discussed. Verrucae were present on the pulmonic valve in over 40 per cent of the cases in Groups IV and II. Their incidence was somewhat lower in the pulmonic valves in Groups III and I. They were rare in Group V and not present in Group VI.

The histological structure of the verrucae suggests that they are made up of hyaline eosinophilic material resulting from necrosis and fusion of inflammatory exudate as well as of the superficial layers of the valve. Their appearance is apparently greatly influenced by the clinical course of the disease. Thus, they were fresh and showed considerable reaction at the base in Group I. In Group II they were broad and presented evidence of some organization and considerable reaction at the base. In Group III they were broad, showed increasing incidence of organization with, however, reaction still present at the base. In Group IV some verrucae appeared to consist of eosinophilic degeneration of the superficial layers of the valve with somewhat milder reaction at the base. In Group V the great majority of verrucous lesions consisted of extremely indolent lesions of the eosinophilic degeneration type, and in Group VI, apart from their rarity, they were completely bland and were obviously of the eosinophilic degeneration type. There may be some question as to whether or not lesions of this type should be properly considered verrucae. However, all gradations may be found between the typical extrusion lesions with pronounced inflammatory base seen in active rheumatic fever cases and those consisting solely of degenerating collagen. It does not seem advisable, therefore, to make a sharp differentiation between these lesions. In the healed cases (Group VI) these lesions

may become the seat of a secondary non-bacterial thrombotic deposit which may reach considerable size. Such secondary thrombotic verrucae have been included in the purely descriptive classification of non-bacterial thrombotic endocarditis (Gross and Friedberg<sup>52</sup>). Obviously, therefore, the latter designation does not exclude a rheumatic origin for the underlying condition. When definite evidence of rheumatic stigmata are present in the valves and elsewhere (auricle, rings, pericardium, and so on) the condition should be termed a rheumatic lesion, healed (Group VI) with or without superimposed non-bacterial thrombotic endocarditis.

As already shown, the proliferative and necrotic processes which are concerned with the formation of verrucae occur with predilection at the most exposed portions of the valve (closure line) as well as within culs-de-sac in which eddies or blood stasis may be present, *e.g.*, in the valve pockets and chordae tendineae insertions. It was further shown that verrucae tend to localize at the most distal portion of the valve that still presents inflammatory changes. This was best exemplified in the pulmonic cusps. As deformity of the valve tip takes place, different portions of this structure become the most conspicuous edge presented to the blood stream. As a consequence, fresh rows of verrucae are found on the newly exposed portions. Thus, in the chronic cases it was not unusual to find several distinct rows of verrucae. The oldest and generally completely organized row represented the original closure line. The other rows showed various grades of organization, the most recent and freshest verrucae being situated on what would correspond to the new closure line of the altered valve, even though no actual apposition of these parts may take place because of the stiffening and deformity. This observation immediately throws out of account any consideration which assumes that anatomical and architectural factors in the vascularization of the valve determine the site of formation of these verrucae. Indeed, as was shown particularly in Groups V and VI, such verrucous transformation may take place in valves that are completely devoid of blood vessels but which show evidence that a toxic or irritative process extended from the ring through the valve leaflet into the tip. Furthermore, the mechanism described explains the occurrence of verrucae in the valve pockets and on the chordae tendineae insertions, areas that are unquestionably free from blood vessels normally.

On comparing the incidence of verrucae in the several valves and including in these figures verrucae occurring in the pockets and on the chordae tendineae insertions, it is seen that the highest incidence was in the tricuspid valve and posterior mitral leaflet. The incidence of these lesions on the aortic valve and anterior mitral leaflet, however, was so close to this that the differences could be accounted for on a statistical basis. Clearly, however, the pulmonic valve showed the lowest incidence of verrucae. It is of interest to note that the incidence of ring and valve lesions was also decidedly lower in the pulmonic valve.

The extent of the deformity varied considerably in the several valves, being greatest in the mitral and next in the aortic, tricuspid and pulmonic, in this order. These differences occurred in spite of the fact that the acuity of the initial lesion (ring lesion) and the continued activity (incidence of verrucae) appear to be approximately the same in each of these three valves. It is interesting to speculate on the determining factors that lead to these marked differences in the subsequent development of the valvular lesion. That vascularization of the valves plays no rôle in this, is obvious for the following reasons: (1) valvulitis may occur in totally non-vascularized valves; (2) observations by Gross and Kugel,<sup>47</sup> which have been recently extended<sup>50</sup>, indicate that normal valves rarely, if ever, possess blood vessels distal to the rings; (3) recent workers who have claimed a high incidence of vascularization in normal valves could show no parallel between their figures and the incidence of valvular lesions<sup>51</sup>; and (4) once a rheumatic process has set in, the incidence of valve vascularization is practically universal, certainly in the first five clinical groups discussed. Nevertheless, the extent of valvular damage, as is well known, differs in the several valves of the heart. On the other hand, many observations indicate that the pressure to which the valve leaflet is exposed may be an important factor in determining the extent of the valvular deformity produced. The additional trauma component of intracardiac pressure, in the presence of the rheumatic infection, can account for the considerably greater extent of the valvular deformities that occur in the left heart. The trauma of valvular apposition may explain the predilection of the valve tips as opposed to the proximal two-thirds of the leaflet for the localization of the continued fibroblastic proliferative process. Of considerable interest in this connection is the frequency

with which the tricuspid valve is the seat of verrucae in the presence of hypertension of the lesser circulation (pulmonary emphysema, mitral stenosis, and so on), even in the absence of fresh lesions on the mitral valve. The fact that the tricuspid valvular lesion is generally far more severe than the pulmonic may be accounted for by the continued infection of the tricuspid ring through the annulus extensions from the aortic root and from the mitral valve. As already shown, the pulmonic ring is not linked to these annulus extensions.

In further support of the view that intracardiac tension probably plays an important rôle in determining the progress of an initial rheumatic lesion are the figures reported by Gross and Ehrlich<sup>40</sup> on the incidence of Aschoff bodies in the several chambers of the heart. They showed that Aschoff bodies occurred with greatest frequency in the left ventricle, and with decreasing frequency in the right ventricle, left auricle and right auricle, in that order. This incidence of Aschoff bodies is strikingly paralleled by the tension within the respective chambers. These authors also showed that there may be differences in the incidence of Aschoff bodies within various parts of the same chamber. This suggests that there are additional factors determining local predisposition, of which we have at present no knowledge. It is not necessary to assume that the epithelium covering valve tips has especial phagocytic properties since the pressure factors discussed above can adequately explain the localization of particles (bacteria) at these sites. Finally, differences in the oxygen tension of the blood bathing the valves in the right and left hearts may also have some bearing on the progress of the lesions.

The striking differences in the inflammatory reactions within the valves as influenced by the clinical course of the disease bring up the question as to what rôle general or local tissue immunity may play in this connection. It was shown that if a patient dies during a first attack or if he suffered from only one previous attack within 1 year previous to the fatal outcome, the lesions are flagrant and differ chiefly in the nature of the blood vessels, the latter in turn being determined by the duration of the rheumatic bout. However, in the more chronic cases the inflammatory reaction is considerably subdued. It may be argued that the severity of the disease which is sufficient to lead to a fatal outcome during the first or second attack



occurring within 1 year, may be so intense that flagrant exudative phenomena are to be expected. Similarly, the fact that a patient can survive a series of rheumatic attacks or present a chronic course, may be indicative of less severe infection, *i.e.*, one that would lead to a more indolent type of reactivity in the valve. On the other hand, these differences in the severity of the reaction may indicate differences in the relative immunity of the patient to the disease. Indeed, the clinical groups into which the rheumatic cases were divided may be considered as representing various degrees of resistance to the infection. Group I represents a fulminating infection with little or no resistance. In Group II, one attack was successfully resisted for a short time only, the patient dying of either a second attack or a second exacerbation. In Group III, the first attack was even more successfully resisted; however, after a period of 2 or more years, a second attack encountered insufficient resistance and led to death. Group IV can be construed as representing partial immunity to the disease, the patient successively showing temporary phases of greater or lesser resistance to the infection. In Group V, immunity is great enough to permit of a chronic drawn-out course, without, however, complete healing. Group VI represents complete immunity to the infection with cessation of all activity. Considered in this light, the qualitative and quantitative differences in the inflammatory phenomena as observed in the various groups may well depend, at least to some extent, on such differences in immunity or reactivity. In previous reports it was shown that similar differences, corresponding to the clinical course of the disease, may be observed in the response to the rheumatic infection of the myocardial interstitium (Aschoff bodies<sup>40</sup>), coronary tree,<sup>41</sup> left auricle<sup>43</sup> and pericardium.<sup>45</sup>

The comparison of the valvular lesions occurring in the different clinical groups has already been presented in the summaries given at the end of each of these chapters. It need only be repeated that the first two groups are notable for the exudative phenomena, and the last four groups for the productive changes. Group IV occupies an intermediary position in that it represents at the same time the chronicity of the latter groups, as well as the acute exacerbations of the former. Group VI represents complete healing of the rheumatic process or cessation of the rheumatic lesions. As such, the findings in this latter group are of extreme importance in that they disclose

the stigmata of the completely involuted rheumatic processes in the valve leaflets.

In conclusion, attention should be drawn to the additional gross lesions described in this report. Until comparatively recently the only gross rheumatic lesions known to occur in the heart were those due to the acute and healing stages of pericardial inflammation, the fresh and healed verrucae on the closure line and chordae tendineae insertions of the valves, the valvular deformities with the characteristic commissural agglutinations of the aortic cusps, the thickening of the chordae tendineae, the regurgitant endocardial pockets (also occurring in other conditions), the auricular endocardial lesions and, rarely, the macroscopic Aschoff bodies. To these there have been recently added the lesions at the roots of the great vessels (Gross<sup>42</sup>) which produce dimpling in the sinus pockets (Fig. 10) and the thickening and prominence of the subvalvular angles and ring regions (Gross and Friedberg<sup>46</sup>). In the present communication, descriptions are given of the more minute topographical changes found in the valves, including elongation of the auriculoventricular leaflets, with obliteration of their normal scalloping, the ham shaped chordae tendineae insertions, the approximation of the semilunar folds of the semilunar cusps to the free edges and their disappearance, the notching, entropion and ectropion of the semilunar cusps, the characteristic pocket lesions consisting of verrucous, polypoid and nodular formations, and the agglutinations and rounding of the auriculoventricular valve pockets with obliteration of their sharp angle. A description of the pathogenesis of these lesions, their life cycles and their incidence in the various clinical subdivisions of rheumatic fever is given.

#### SUMMARY AND CONCLUSIONS

There have been described in this report the incidence and gross and microscopic appearance of lesions in the valves, valve pockets and chordae tendineae occurring in 97 cases of rheumatic fever. These cases have been divided into six clinical groups which represent various courses taken by this disease. It has been shown that each group presents certain gross and microscopic features that bear a relation to the clinical grouping. Anatomical evidence is presented which suggests that the course taken by the disease as well as the response of the tissue may be determined by the relative state of

immunity. This does not, however, imply that rheumatic fever is primarily an allergic disease. New macroscopic and microscopic data are presented on the development of the rheumatic lesions in the valves, and a discussion is given of the factors which determine the spread of infection, the localization of the verrucous and other lesions, the extent of the valvular damage and the pathogenesis of the characteristic deformities of the valvular apparatus. Certain stigmata of the rheumatic process occurring in completely healed valves are described. These supply additional data which are of value in elucidating the pathogenesis of other cardiac lesions. A description is also given of the changes that take place in non-rheumatic valves during the first eight decades of life.

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## DESCRIPTION OF PLATES

### PLATE 146

FIG. 1. Cross-section of normal posterior mitral leaflet. Age 8 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = auricularis layer discernible only as a delicate elastic lamella; B = spongiosa layer; C = fibrosa layer; D = valve ring; E = valve pocket; F = gelatinous valve tip; G = first order chorda tendinea insertion; H = left auricular endocardium; I = left auricular myocardial wedge; J = left auricular pericardial wedge; K = left ventricular myocardium; L = columna carnea.

FIG. 2. Cross-section of normal posterior aortic cusp (to the right of the lower arrow point) and normal anterior mitral leaflet (to the left of the upper arrow point). Age 8 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = proximal layer of valve; B = spongiosa layer; C = fibrosa layer; D = valve ring; E = aortic valve pocket; F = valve tip; G = second order chorda tendinea insertion; H = left auricular endocardium; I = left auricular myocardial wedge; J = left auricular pericardial wedge; K = aortic root; L = intervalvular fibrosa.

FIG. 3. Gross photograph of tricuspid valve from a case of active rheumatic fever (Group I), showing chordae tendineae agglutinations to underlying endocardium. Age 9 years.

A = right auricle; B = anterior tricuspid leaflet; C = retracted septal tricuspid leaflet; D = chordae tendineae agglutinated to ventricular wall; E = outflow tract of right ventricle.

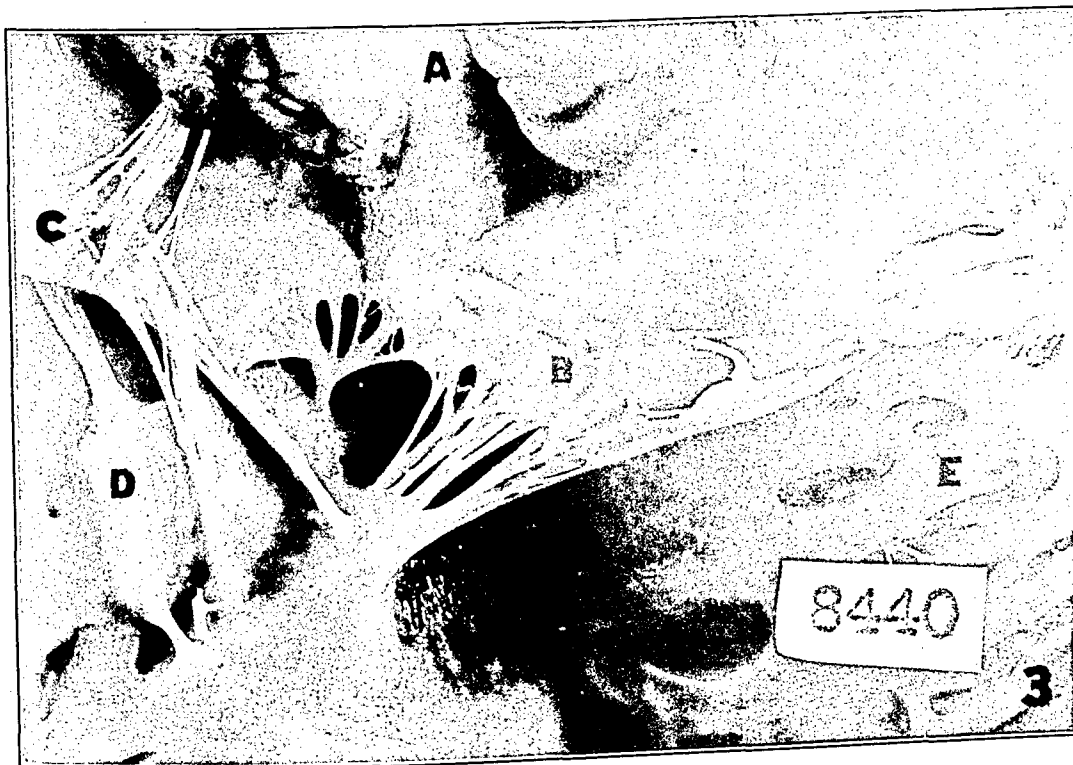
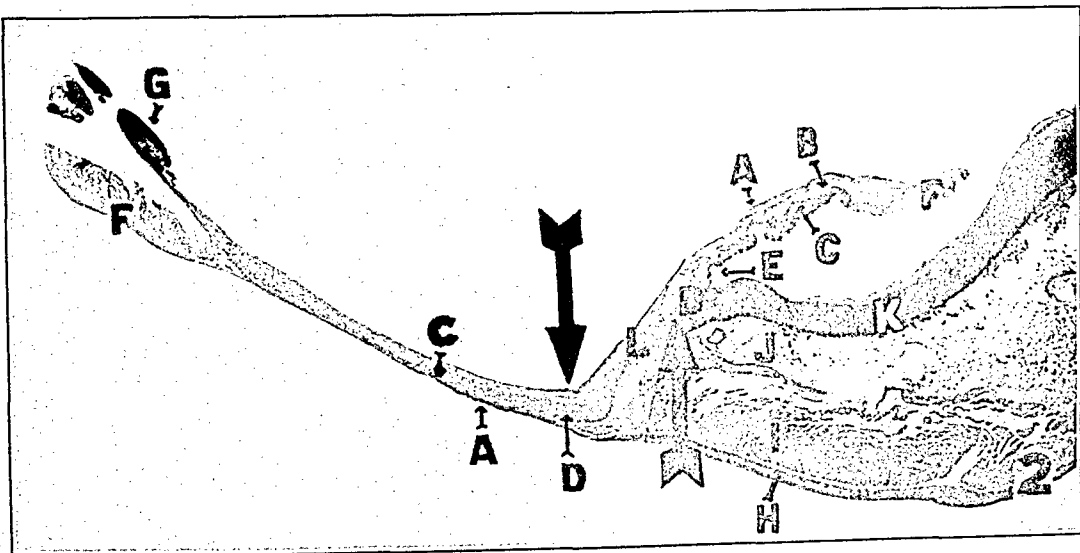
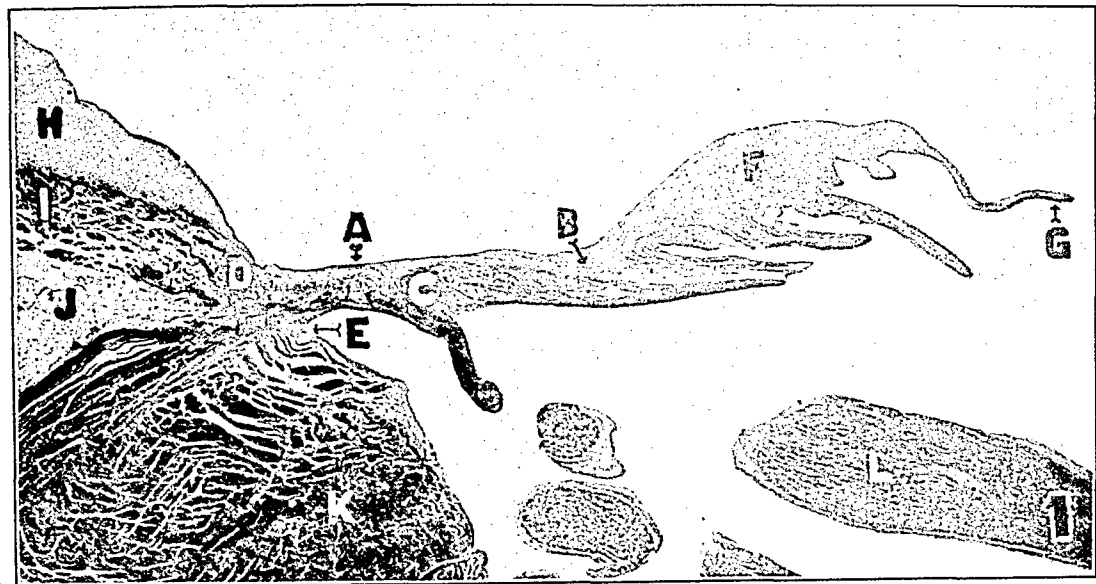




PLATE 147

FIG. 4. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group I). Age 17 months. Low power. Hematoxylin and eosin stain.

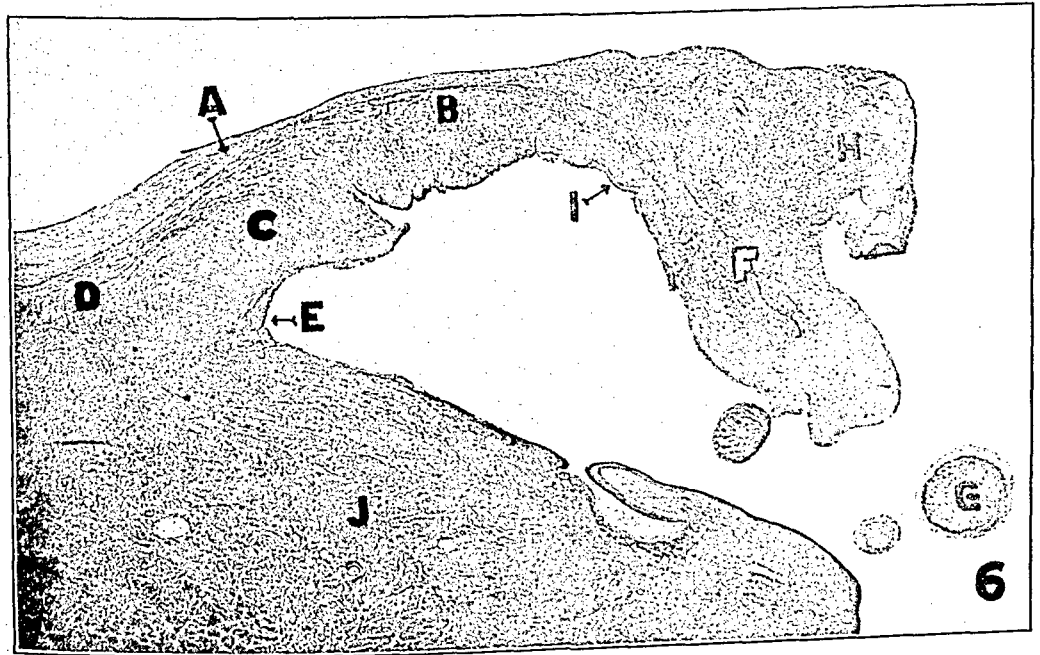
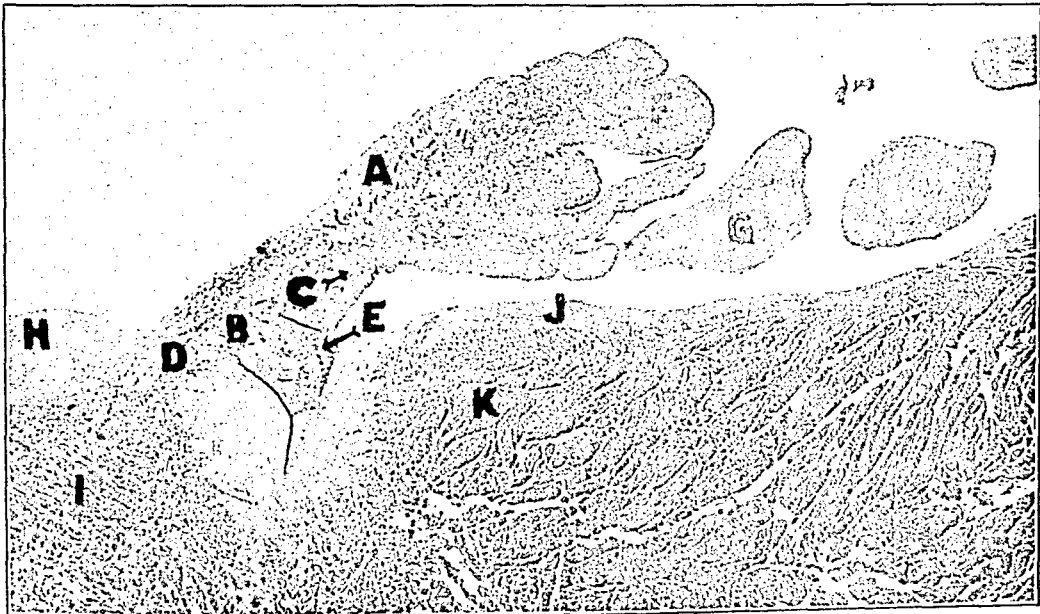
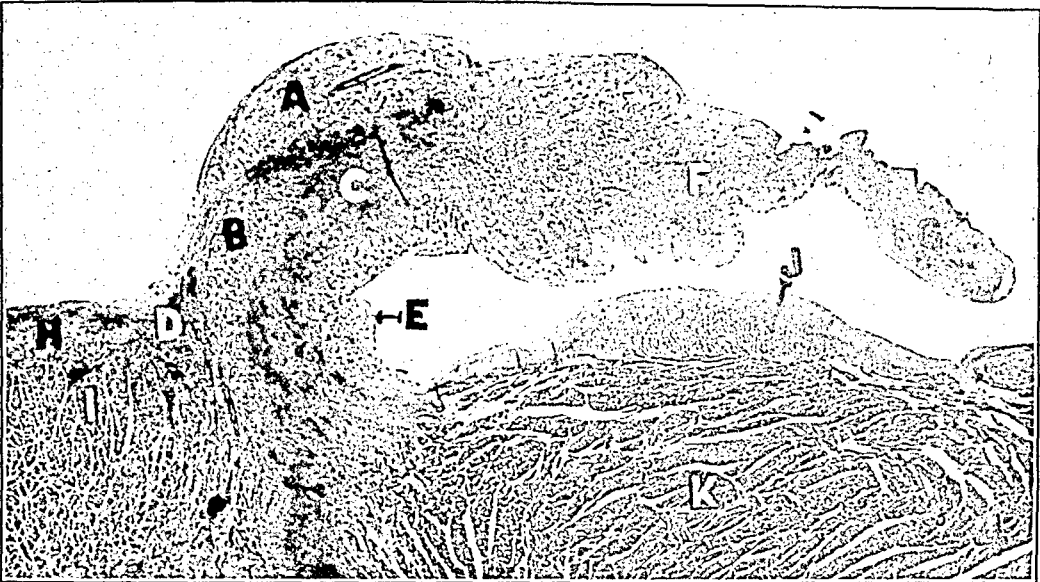
A = edematous inflamed hypercapillarized auricularis layer; B = inflamed hypercapillarized spongiosa layer; C = inflamed capillarized fibrosa layer; D = inflamed valve ring; E = inflamed capillarized pocket reduplication becoming continuous with inflamed capillarized arterialis reduplication; F = inflamed valve tip; G = inflamed, capillarized, thickened first order chorda tendinea insertion; H = inflamed left auricular endocardium (note inflamed subendocardium); I = left auricular myocardial wedge; J = left ventricular endocardial thickening; K = left ventricular myocardium.

FIG. 5. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group I). Age 2½ years. Low power. Hematoxylin and eosin stain.

A = edematous inflamed hypercapillarized auricularis layer; B = inflamed capillarized spongiosa layer; C = inflamed capillarized fibrosa layer; D = inflamed valve ring; E = inflamed capillarized valve pocket; F = inflamed fibrotic valve tip with verrucous change; G = inflamed chordae tendineae agglutinated by verrucous material; H = inflamed left auricular endocardium (note inflamed subendocardium); I = left auricular myocardial wedge; J = inflamed thickened left ventricular endocardium; K = left ventricular myocardium.

FIG. 6. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group I). Age 6½ years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inflamed widened auricularis layer; B = edematous spongiosa layer; C = fibrosa layer; D = valve ring; E = inflamed reduplication in valve pocket; F = inflamed valve tip; G = cross-section of chorda tendinea showing reduplication of the endocardial covering; H = verrucous material with granulation tissue base situated on closure line (note delicate layer of fibrin attached to the surface of the verrucae); I = very delicate ventricular reduplication; J = left ventricular myocardium.



# PLATE 148

FIG. 7. Cross-section of tricuspid leaflet from a case of active rheumatic fever (Group II). Age 13 years. Low power. Hematoxylin and eosin stain.

A = widened vascularized auricularis layer (note numerous intimal musculo-elastic hyperplastic vessels); B = vascularized spongiosa layer; C = fibrosa layer; D = vascularized valve ring; E = vascularized fibrotic reduplication in tricuspid pocket; F = fibrotic vascularized valve tip; G = first order chorda tendinea insertion (note fibrotic reduplication); H = left auricular endocardium; I = left auricular myocardial wedge; J = left auricular pericardial wedge; K = left ventricular myocardium; L = thickened third order chorda tendinea insertion.

FIG. 8. Cross-section of right aortic cusp from a case of active rheumatic fever (Group II). Age 18 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastified ventricularis reduplications; B = widened fibrotic vascularized spongiosa layer; C = fibrosa layer; D = fibrotic valve ring (note multiple elastified subaortic reduplications with intimal musculo-elastic hyperplastic vessels); E = aortic valve pocket showing polypoid formation; F = fibro-elastic transformation of valve tip with approximation of greatly thickened ventricularis layers to fibrosa and compression of spongiosa; G = fibrotic arterialis reduplication; H = verrucous lesion on closure line; I = aortic root; J = ventricular myocardium; K = considerably thickened blood vessel showing intimal musculo-elastic hyperplastic lesion.

FIG. 9. Cross-section of right aortic cusp from a case of active rheumatic fever (Group II). Age 14 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = multiple elastified ventricularis reduplications continuous with subaortic reduplications; B = fibrotic vascularized spongiosa layer; C = fibrosa layer; D = fibrotic valve ring (note multiple elastified subaortic reduplications with numerous intimal musculo-elastic hyperplastic vessels); E = aortic ring annulus permeated with intimal musculo-elastic hyperplastic vessels; F = fibro-elastic transformation of valve tip showing earliest stages of entropion; G = aortic valve pocket; H = verrucous lesion surrounding valve tip; I = aortic root; J = retroaortic pericardial mantle (note intimal musculo-elastic hyperplastic vessels); K = left auricular myocardial wedge. L = left auricular endocardium.

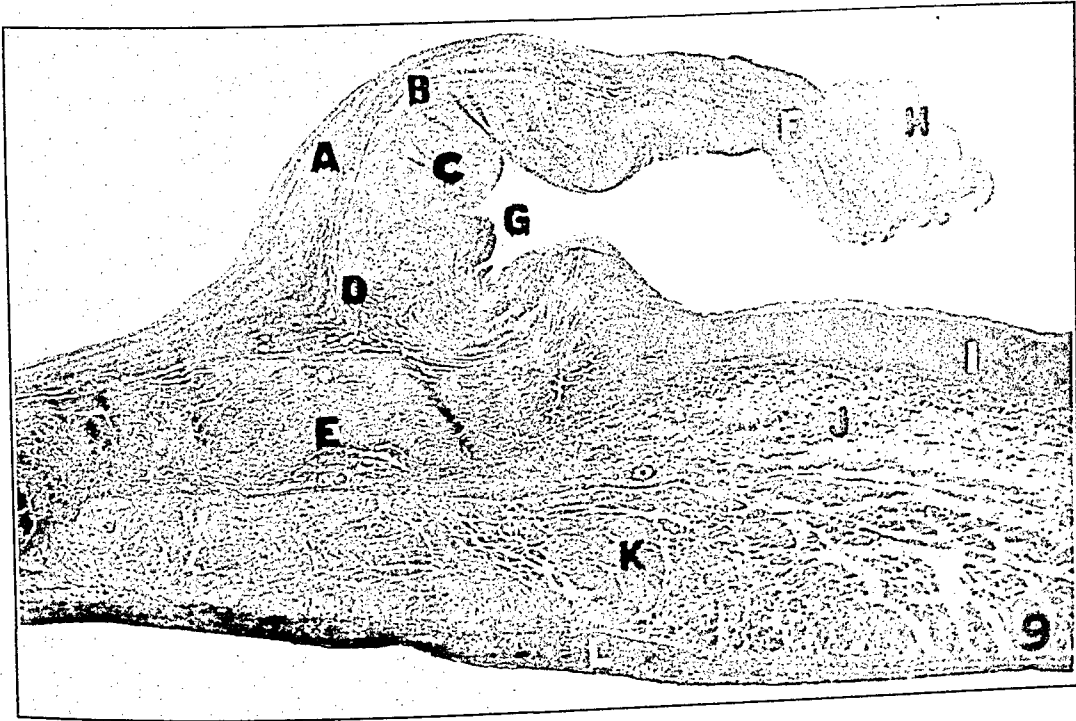
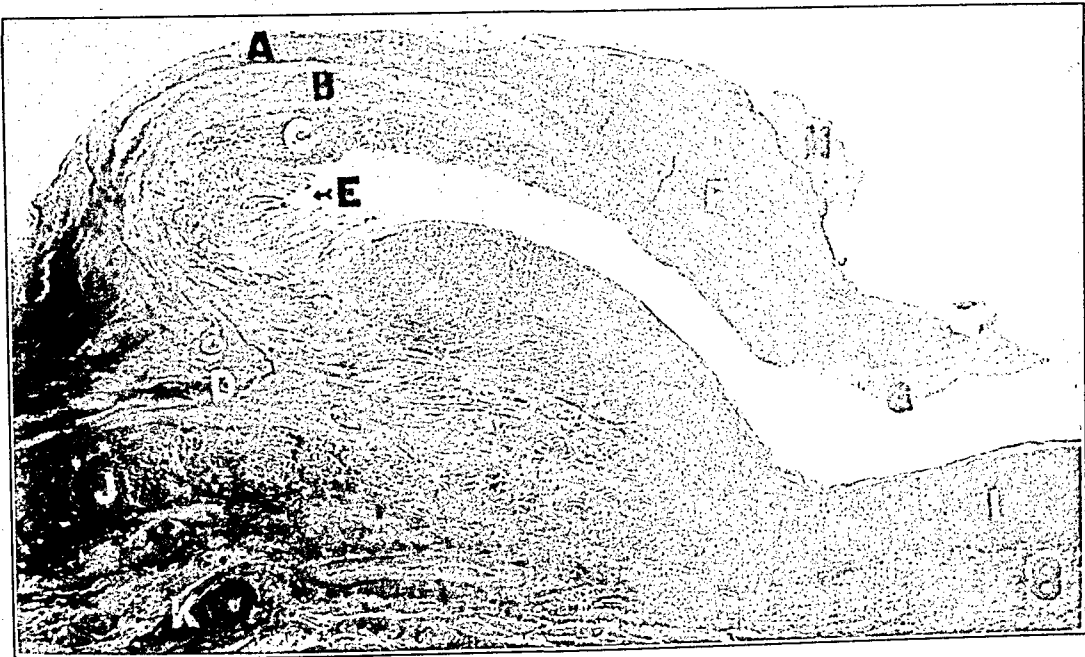
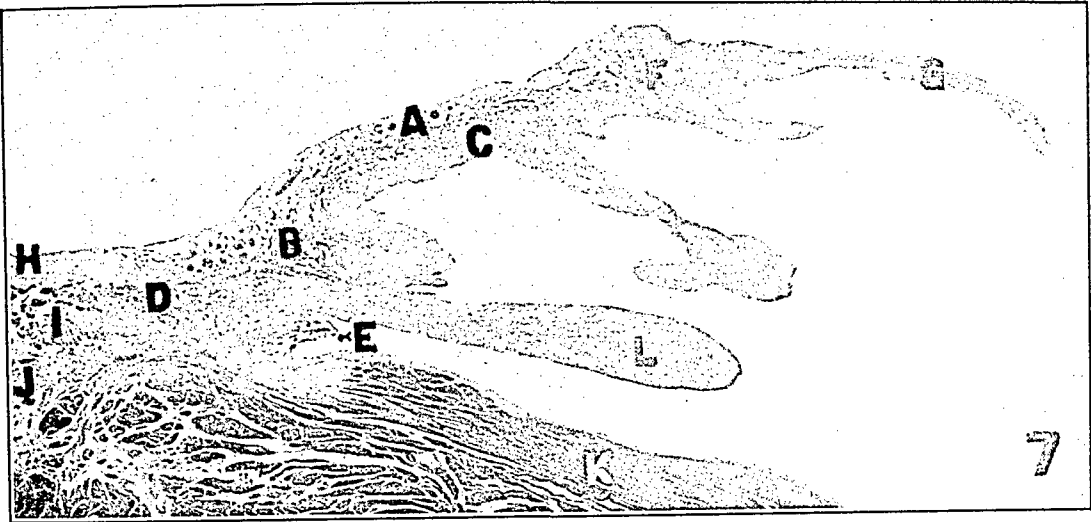


PLATE 149

FIG. 10. Gross photograph of aortic valve from a case of active rheumatic fever (Group IV). Age 12 years.

A = aorta; B = left coronary ostium; C = right coronary ostium; D = posterior sinus pocket (note dimpling of annulus); E = right aortic cusp (note notching at center of free margin and approximation of (F) semi-lunar folds to free margin); G = posterior aortic cusp (note rolling and thickening of free edge, beginning entropion and approximation of semi-lunar folds to free margin); H = irregularity of subaortic angle due to formation of subaortic angle lesions; J = bridges of verrucous material agglutinating commissure; K = ventricular aspect of anterior mitral leaflet; L = outflow tract of left ventricle.

FIG. 11. Gross photograph of mitral valve from a case of active rheumatic fever (Group IV). Age 49 years.

A = left auricle; B = anterior mitral leaflet (note gross vascularization of body of leaflet, also fresh verrucae along closure line); C = verrucae situated on chordae tendineae insertions (note ham shaped terminations of latter); D = posterior mitral leaflet with marked straightening of scalloped edge (note verrucae on closure line); E = posterior papillary muscle; F = anterior papillary muscle.

FIG. 12. Cross-section of right aortic cusp from a case of active rheumatic fever (Group IV). Age 13 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = thick fibrotic vascularized ventricularis reduplications; B = elastified compressed spongiosa layer; C = fibrosa layer; D = fibrotic valve ring (note distorted compressed capillaries); E = fibro-elastified reduplication in aortic pocket; F = ectropion of fibro-elastified valve tip; G = verrucae on new closure line; H = verrucae in cul-de-sac; I = aortic root; J = ventricular myocardium showing considerable scarring.

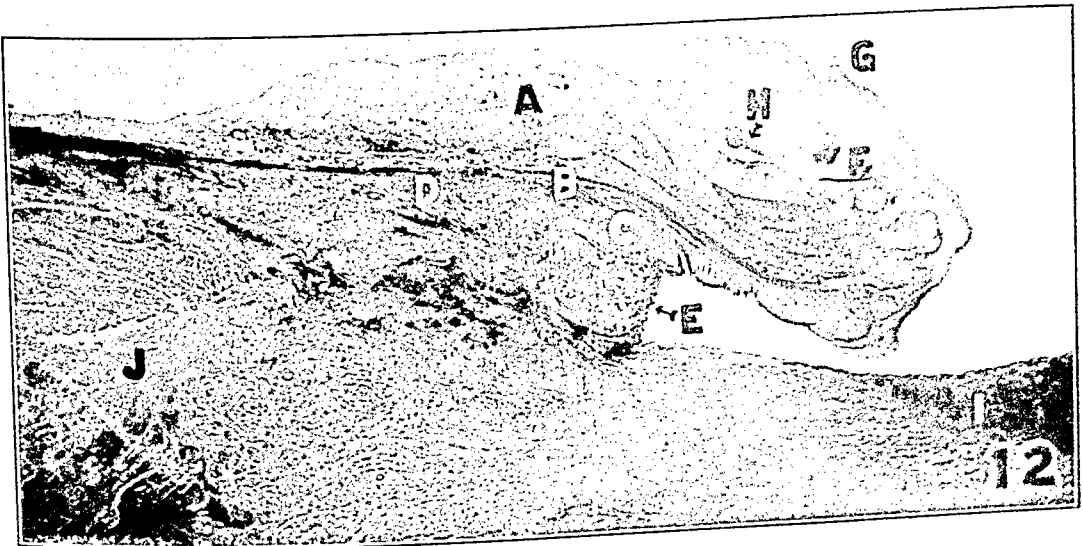
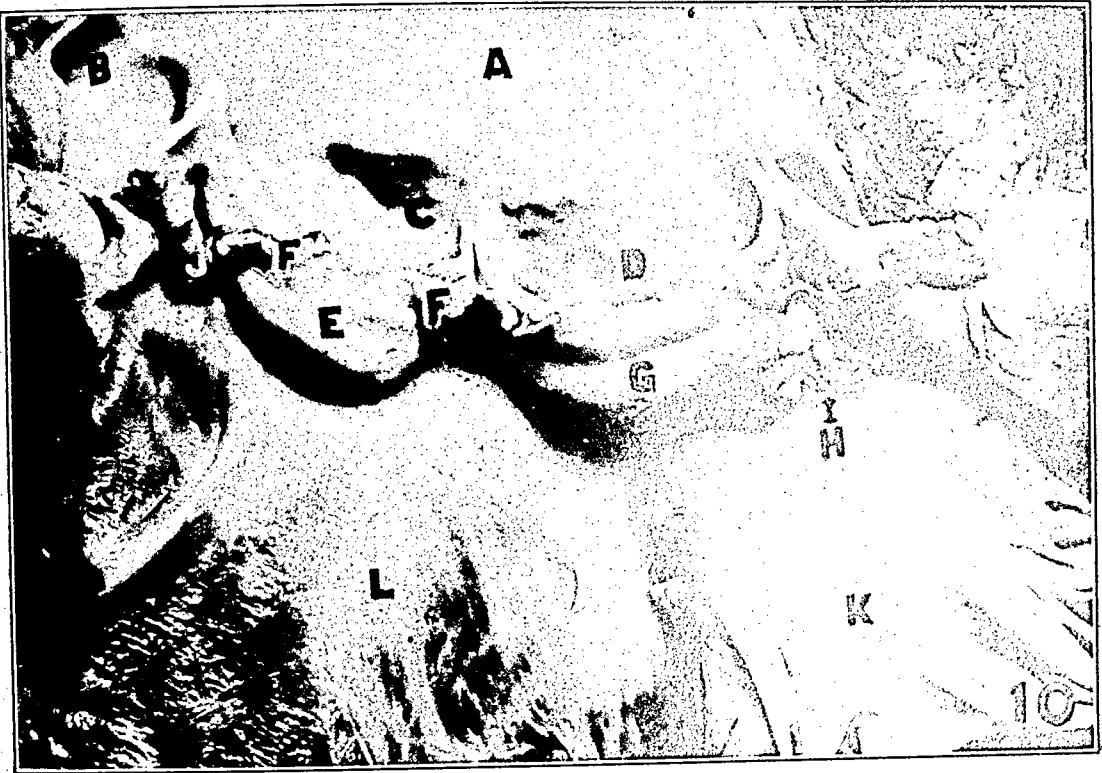


PLATE 150

FIG. 13. Cross-section of anterior mitral leaflet from a case of active rheumatic fever (Group V). Age 33 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

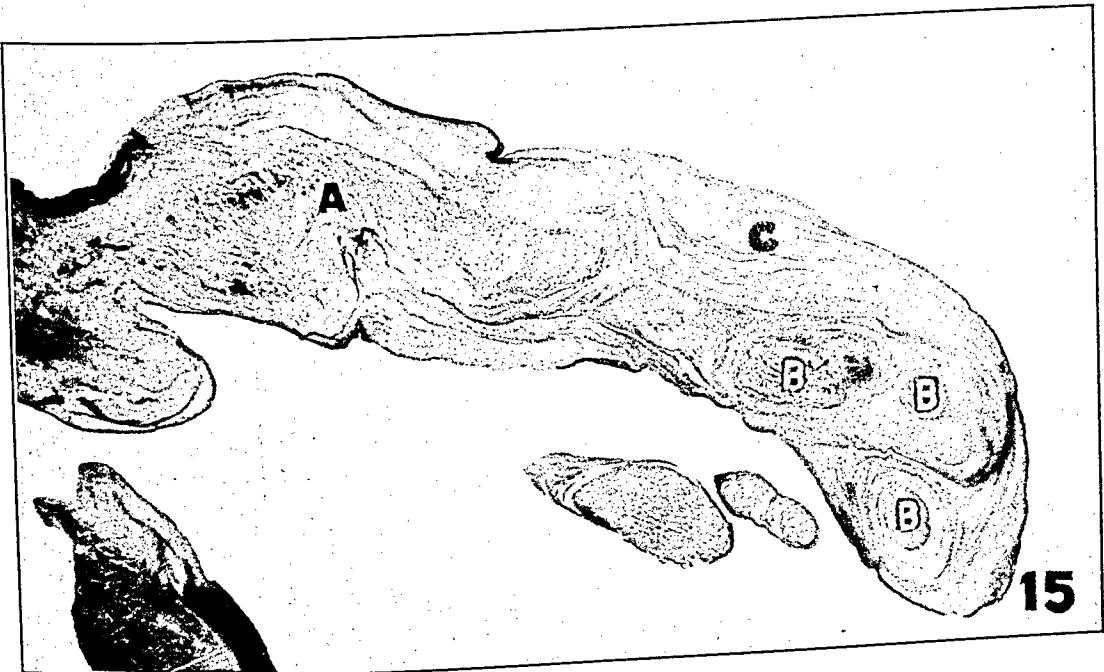
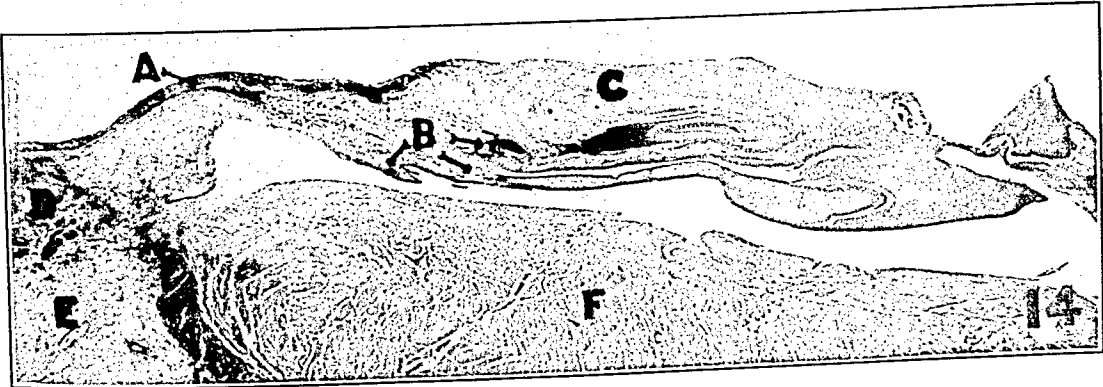
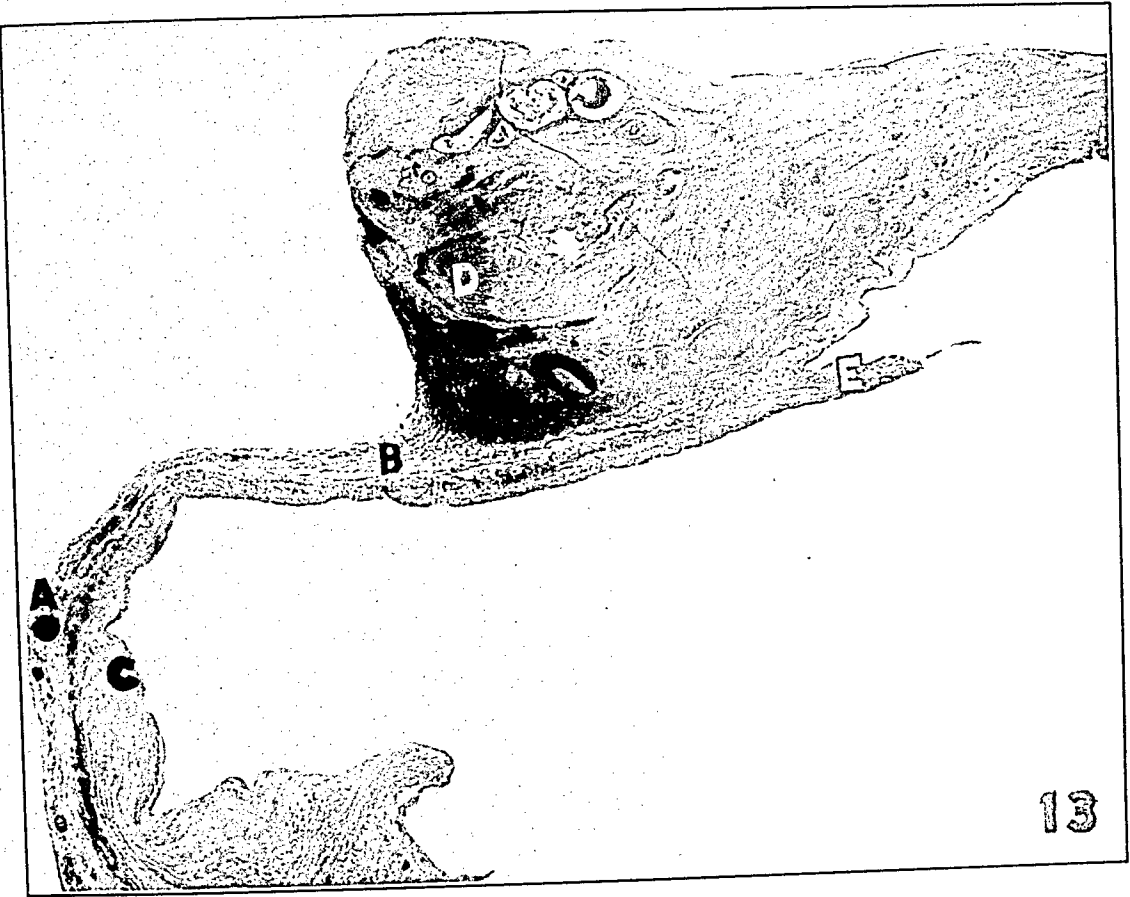
A = superficial vascularization (injected) of auricularis layer in the proximal two-thirds of the valve leaflet; B = moderately widened spongiosa layer; C = fibrosa layer; D = enormously thickened distal third of the valve leaflet. The thickening is due to fibro-elastification and fusion of auricularis and spongiosa layers together with the production of redundant elastic collagenous tissue. Note extensive vascularization of thickened tip. E = chorda tendinea insertion.

FIG. 14. Cross-section of posterior mitral leaflet from a case of active rheumatic fever (Group V). Age 59 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = fused auricularis and spongiosa layers; B = chordae tendineae insertions marking the original site of the valve tip; C = enormously redundant fibrotic tissue from fused auricularis and spongiosa layers spreading over chordae tendineae and producing elongation of the valve leaflet (note absorption of chordae tendineae into this mass); D = left auricular myocardial wedge; E = left auricular pericardial wedge; F = left ventricular myocardium.

FIG. 15. Cross-section of tip of anterior mitral leaflet showing absorption of chordae tendineae and the mechanism of elongation of the leaflet. Case of active rheumatic fever (Group V). Age 66 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = tip of leaflet; B = absorbed chordae tendineae insertions; C = redundant fibro-elastic tissue from fused auricularis and spongiosa layers enveloping chordae tendineae.







# THE OCCURRENCE OF TUMORS OF THE CENTRAL NERVOUS SYSTEM IN ROUTINE AUTOPSIES\*

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## INTRODUCTION

When one considers the rapidly increasing interest, both surgical and pathological, in the subject of tumors of the central nervous system during the past decade it seems truly surprising that so few surveys of a general nature have appeared. Save for Cushing's monograph,<sup>1</sup> and the earlier work of Bailey and Cushing<sup>2</sup> on which the classification at present prevailing is based, published work has dealt almost entirely with special groups of tumors, or with the clinical syndromes caused by tumors of particular localization.

From the point of view of the general pathologist something appears desirable to bridge the gap between this work of the specialist and the ordinary pathological experience of a general hospital. Cushing's great series will remain a rich mine of information for a long time to come. It is subject, however, by its manner of compilation, to certain selective influences. Based as it is on the material of a renowned neurosurgical clinic, it represents a notable preponderance of the "surgical" type of case. Also no attempt is made in it to indicate any estimate of the incidence of tumors of the nervous system in either the hospital or the general population.

Estimates of the incidence of brain tumors culled from various sources show a striking lack of agreement. Some recent surveys of the cancer problem<sup>3,4</sup> ignore cerebral neoplasms entirely. In contrast, perhaps the most generous estimate is that of Bailey and Cushing,<sup>2</sup> who state that next to the uterus the brain is the most common site of tumors. An intermediate position is taken by Ewing,<sup>5</sup> who believes that probably about 1 per cent of all deaths may be caused by tumors of the brain — a not inconsiderable figure. In a subject so notoriously beset by diagnostic pitfalls as is the recognition of intracranial tumors, accurate statistics must await a far more universal surgical exploration or postmortem examination than now prevails. At present we have only random samplings, chiefly in the earlier literature. Ewing<sup>5</sup> quotes a few of these, mostly from German publi-

\* Received for publication March 30, 1936.

cations, indicating that brain tumors were found in from 1 to 2 per cent of all autopsies. At any rate we can agree with Bailey's more recent statement<sup>6</sup> that intracranial tumors are certainly a good deal more common than is usually supposed. In view of the scarcity of general surveys since the introduction of a histogenetic classification, it is felt that a study of the tumors of the central nervous system occurring in the autopsy service of a large general hospital may furnish material of some interest and value.

### MATERIAL AND METHODS

The material for this study was drawn from the autopsies performed at the Boston City Hospital from January, 1896, through December, 1934, a total of 10,592. In 5150 of these the head was examined. Exclusive of the neoplasms of the nervous system 902 malignant tumors were found: 368 benign tumors, chiefly leiomyomas, were noted, though this figure is doubtless much too low as many small masses were probably overlooked.

From the great majority of autopsies slides of the tumors of the nervous system stained with eosin-methylene blue and with phosphotungstic acid hematoxylin were still available in the files of the Mallory Institute. The slides from a moderate number of the early autopsies had unfortunately disappeared. In a few such cases tissue was still available and this was cut and stained to permit of classification of the tumor. In the discussion of the gliomas, when neither tissue nor slides were available such tumors were listed in the unclassified group even though in a considerable number of instances the recorded description was sufficiently clear to make possible a reasonably accurate classification.

On some tumors, encountered in the last 4 years of the series, during the author's period of residence, the Spanish methods of metallic impregnation were employed with occasional helpful results. In general, however, it seemed that impregnations found their greatest utility in Bailey's pioneer work. There they furnished invaluable evidence in proving the similarity of the tumor cells in morphology and staining reaction to the already recognized stages of glial development. Now that the foregoing cell types are fairly generally recognized it is doubtful, save in selected cases, if the considerable labor required by capricious impregnation methods promises sufficient reward for the effort.

TABLE I

Total autopsies .....	10,592		
Heads examined .....	5,150		
Tumors other than those of the central nervous system:			
Malignant .....	902		
Benign .....	368		
Total .....	1,270		
Total tumors of the central nervous system — 188			
I. GLIOMAS .....	81	—	43.1 per cent
Astroblastoma .....	1		
Ependymoma .....	2		
Astrocytoma ....	15		
Glioblastoma multiforme .....	25		
Medulloblastoma .....	10		
Oligodendroglioma .....	1		
Pinealoma .....	1		
Spongioblastoma polare .....	8		
Ganglioneuroma .....	0		
Neuroepithelioma .....	0		
Unclassified gliomas .....	18		
II. PITUITARY ADENOMAS .....	6	—	3.2 per cent
Chromophobe .....	1		
Chromophile .....	4		
Mixed .....	1		
III. SHEATH TUMORS .....	22	—	11.7 per cent
Meningioma .....	18		
Acoustic neuroma .....	4		
IV. METASTATIC TUMORS .....	29	—	15.4 per cent
V. INVASIVE TUMORS .....	4	—	2.1 per cent
VI. BLOOD VESSEL TUMORS .....	5	—	2.6 per cent
Cystic hemangioma .....	2		
Racemose aneurysm .....	3		
VII. CONGENITAL TUMORS .....	8	—	4.3 per cent
Cranopharyngioma .....	0		
Cholesteatoma .....	4		
Chordoma .....	4		
VIII. GRANULOMATOUS TUMORS .....	19	—	10.1 per cent
Tuberculoma .....	16		
Gumma .....	3		
IX. SPINAL CORD TUMORS .....	4	—	2.1 per cent
X. UNCLASSIFIED TUMORS .....	10	—	5.3 per cent

As a routine method by far the best results were obtained by immediate Zenker fixation of representative blocks of fresh tissue, followed by staining with not overripe phosphotungstic acid hematoxylin. This method proved thoroughly reliable and simple, and stained sharply the nuclei, cytoplasmic outline, glia fibrils and blepharoplasts. When it was desired to bring out in greater contrast the vasculature and mesodermal elements Masson's trichrome stain on the same Zenker tissue furnished precise and colorful prepara-

tions. The prerequisite for satisfactory microscopic material is prompt and efficient fixation of small pieces of fresh tissue. The practice of hardening brains entire and sectioning them subsequently may provide somewhat neater gross museum specimens, but the price of exhibits so prepared is the irrevocable loss of all finer histological detail. If a gross specimen is desired it may be obtained by making one clean incision bisecting brain and tumor in the desired plane. One half may be preserved intact for the museum, while the remaining tissue is cut into pieces suitable for histological fixation.

The material for discussion is grouped in Table I. Save for minor differences the arrangement conforms to that in Cushing's monograph.<sup>1</sup> Following the precedent set by Cushing the granulomas of tuberculosis and syphilis were included with the tumors of the nervous system, though not with those of other organs. Subsequently each group of tumors will be discussed separately.

## THE GLIOMA GROUP

### *Astroblastoma*

Only 1 example of this rather questionable tumor entity occurred in this series. The patient, a 49 year old female, entered the hospital complaining of cerebral symptoms of about 2 months duration, and died suddenly and unexpectedly while under observation. Autopsy revealed in the white matter of the right occipital lobe a thin walled, sharply demarcated cystic tumor, 5 cm. in diameter, filled with clear colorless fluid. The tumor pushed forward the right corpus striatum and the posterior horn of the right lateral ventricle. The wall of tumor tissue was 1 to 3 mm. thick, soft and light brown in color, with prominent small vessels coursing through it.

Microscopically the tissue was composed of a thin mat of bulky cells radiating around and attached to the numerous vessels by short stout sucker feet. The vessels had moderately thick collagenous walls and prominent, actively proliferating endothelium. Small areas of the tumor were necrotic. Mitoses were fairly numerous and glia fibrils were very scanty. Next to the brain tissue the tumor cells were smaller and their cytoplasm poorly preserved. There was no evidence of invasive growth, and the line of demarcation between tumor and brain was bordered by a narrow zone of reactionary gliosis.

This single case gives little support to the existence of the astroblastoma as a separate variety of glioma. Bailey<sup>6</sup> admits that the group is small and ill-defined. The cells of this tumor undoubtedly were predominantly of astroblastic form, but such cells in small groups are noted in other gliomas, and the extensive cystic degeneration in this case tends to corroborate the view of Roussy and Oberling,<sup>7</sup> who regard the astroblast-like tumor cells as degenerating forms of neoplastic astrocytes.

### *Ependymoma*

Two examples of the tumor arising from ependymal cells appeared in this series, and though the number was small, the proportion of the total gliomas was almost identical with that in Cushing's<sup>1</sup> large collection. The 1st tumor, in a female of 20 years, arose apparently in the right lateral recess of the fourth ventricle. It extended laterally to appear beneath the meninges covering the cerebello-pontine angle and thence spread as a flattened mass anteriorly to the pons, posteriorly along the medulla, and for a short distance upward over the cerebellar surface.

In the 2nd patient, a male negro 40 years of age, a large, partly cystic tumor occupied the inferior half of the right frontal lobe, extending to the meninges, and producing some erosion of the underlying orbital roof.

Microscopically both tumors were purely of the spindle cell spongioblastic type, originally designated ependymoblastoma by Bailey and Cushing.<sup>2</sup> In both tumors the cells radiated characteristically about the numerous small vessels. Glia fibrils were not formed, but the cell tails stained heavily and, especially in the 2nd case, numerous blepharoplasts could be seen. In spite of their reputation for slow growth mitoses were fairly numerous in the 2nd tumor. Considerable necrosis was present but no calcification. The extension to the meninges in both instances, and the bone erosion in the 2nd, appear from the literature to have been quite unusual phenomena.

In neither case could the duration of symptoms definitely be stated. The 1st patient, after 3 days illness, was admitted unconscious and moribund with a diagnosis of tuberculous meningitis. On the 2nd patient brain tumor had been diagnosed 2 years previously at Cushing's clinic, but he refused to consent to operation and was finally admitted to the hospital comatose and in extremis.

*Astrocytoma*

In this series the astrocytoma formed the second largest single group of gliomas, and 15 examples were available. Proportionately they were only about two-thirds as numerous in this collection as in Cushing's<sup>1</sup> series, in which they constituted the largest single division of the gliomas. This difference in incidence may in part be explained by the inherent difference between surgical and postmortem material. Because of their more typical and leisurely evolution of symptoms, one might expect that astrocytomas would be relatively more often diagnosed in the operable stage and referred to a neurosurgical clinic, there to be successfully operated on and included in surgical statistics. As will again appear, this sort of natural selection will tend to increase the ratio of slowly growing and more benign tumors in neurosurgical figures and leave to the general hospital a greater proportion of rapidly growing, atypical and malignant tumors, unrecognized by the general practitioner and so not referred to a specialist.

Of the 15 astrocytomas studied, 1 was in the olivary region in a 6 year old child, 3 were cystic tumors in the cerebellum of young female patients 16 to 35 years of age, and the remaining 11 were in the cerebrum, about evenly distributed in the various lobes. All but 1 of the patients in the latter group were males, and they were considerably older, the average age being 55 years.

Microscopically the tumors of the brain stem and the cerebellum were composed entirely of well differentiated fibrous astrocytes forming abundant coarse glia fibrils. No mitoses were seen and the manner of growth was entirely expansile.

The cerebral astrocytomas were histologically a much less homogeneous group. An attempt to divide them into protoplasmic and fibrillary varieties failed, because while their cells were almost all fairly uniform, bulky and closely packed, resembling protoplasmic astrocytes, on closer inspection more or less abundant glia fibrils were invariably to be seen. Hence it seemed best to regard all of these astrocytomas of the cerebrum as tumors of mixed cell type with a preponderance of the protoplasmic form of cell. In all cases the tumors were moderately vascular, necrosis was quite extensive and hemorrhage was frequent.

In both their gross character and their microscopic appearance these cerebral astrocytomas tended to merge insensibly into the

glioblastomas, and no doubt many were on their way to assume a frankly malignant nature. However, their type cell was fairly uniform and, as with fibrosarcomas, a perhaps arbitrary division line was established, based on their apparent rate of growth as determined by the presence or absence of mitotic figures.

Viewed as a whole, the astrocytomas seemed to fall into two major divisions. In the cerebellum they occurred in younger patients as grossly cystic tumors of nearly pure fibrillary cell type. In contrast the cerebral tumors, at least in postmortem material, were found in older subjects. Histologically they were all of mixed fibrillary and protoplasmic cell type and were distinguished from the glioblastoma multiforme chiefly on the quantitative basis of slower apparent growth, as indicated by the scarcity of mitoses.

Of the 11 patients with cerebral astrocytomas 6 were diagnosed clinically, and 3 of these were operated on but removal of the tumor was found to be impossible. Three others died with a diagnosis of vascular accident, and on 2 the diagnosis was not noted. Craniotomy was performed on 1 of the 3 patients having a cerebellar tumor. The other 2, and the child with the tumor of the medulla, died soon after admission without a clinical diagnosis having been entered on the autopsy records.

### *Glioblastoma Multiforme*

As suggested previously, autopsy figures, in contrast to surgical statistics, tend to show a relatively larger incidence of the atypical and rapidly growing malignant tumors presenting greater diagnostic difficulties during life. This hypothesis was further substantiated by the finding in this series of 25 cases of glioblastoma multiforme, constituting almost one-third of all gliomas encountered. At that the figure was still too low as perhaps some of the astrocytomas, and certainly a goodly number of the unclassified gliomas, might have been included in this division.

The cases of glioblastoma multiforme formed a quite homogeneous group. They all presented bulky subcortical tumors of the cerebral hemispheres, which often invaded the ventricles and basal ganglia. The tumors usually showed quite extensive gross degenerative changes in the form of necrosis, liquefaction and hemorrhage. Like the sarcomas of other tissues, the details of cell form varied from case to case and in different fields of the same tumor. Glia fibrils



were scanty or absent. The tumors had in common an anaplastic cell type and numerous mitotic figures, and the diagnosis seemed to rest most firmly on these latter two features, rather than on the somewhat inconstant presence of bizarre giant cells that were probably in good part a degenerative phenomenon. The tissue was richly vascular, but the thickening and proliferation of endothelium frequently described did not in this series of tumors seem to be characteristic of, or even particularly prominent in, the glioblastoma multiforme. In some instances, when necrosis had been extensive, the necrotic portion was invaded by abundant new formed connective tissue. One tumor in a younger patient clearly arose as a malignant transformation in a preexisting astrocytoma. In the great majority, however, the tumors seemed to have been malignant from the start.

All but 3 of the tumors occurred in middle aged subjects, being about evenly distributed in number in the fourth, fifth and sixth decades. Fourteen patients were males and 11 females. Brain tumor was diagnosed on 15, and craniotomy was performed on 8 of these. Five patients died with a diagnosis of cerebral hemorrhage and on 4 others the clinical diagnosis was not noted in the pathological records. One case was thought clinically to have been cerebrospinal syphilis.

### *Medulloblastoma*

Ten examples of tumors considered to be medulloblastomas were encountered in this series. Though they constituted a slightly higher proportion of the gliomas than appeared in Cushing's<sup>1</sup> collection, the difference was not great enough to be significant. Seven tumors corresponded quite closely to the classical description. Five were found in children under the age of 10 years. The growth was in the cerebellum in 2 of these and in the thalamic region of the brain stem in 3. Two other cerebellar tumors occurred in patients 22 and 48 years of age.

All the tumors were moderately rapidly growing and were composed predominantly of small round or piriform cells. Formation of rosettes seldom progressed farther than the appearance of small, loose, ball-like aggregations of cells. The tumor cells aggressively invaded the adjacent brain tissue and frequently spread into the meninges.

Clinically, 2 cases were diagnosed as probable meningitis and 4 as

tumor. Note of clinical diagnosis was lacking on 1 case. Surgical removal of the tumor was attempted without success on 3 patients.

The 3 remaining tumors were rather atypical growths found in the striate region of adults 30 to 57 years of age. Cushing<sup>1</sup> appears to have some doubt about the essential nature of these supposed cerebral medulloblastomas of adults. He suggests that they may be akin to the oligodendroglioma as their life history is often quite similar. Our tumors microscopically showed more extensive vascularization and degeneration than was apparent in the tumors from children, and further to corroborate Cushing's suggestion, scattered astrocytes and rare cells resembling immature oligodendroglia could be found. The bulk of the tissue, however, was scarcely distinguishable from that from the younger subjects, and it appeared that on histological evidence these few tumors from adults must be classified with the medulloblastomas.

### *Oligodendroglioma*

Only 1 recognizable case of this rather uncommon variety of glioma appeared in this series. It occurred as a soft purplish mass largely replacing the left basal ganglia in a 13 year old girl. Microscopically the tumor consisted of closely packed, rather uniform round or oval cells, many having the classical appearance of a sharply bordered empty halo about the nucleus. Occasional mitoses and rare giant cells were seen, the latter probably surviving hypertrophied astrocytes. Penfield's second modification of the method of Hortega for oligodendroglia gave a strongly suggestive partial impregnation of the majority of the tumor cells and revealed among them many typical oligodendrocytes. No striking degenerative changes were present.

The clinical course of the patient extended over about 5 years. The symptoms consisted of headache, gradual mental change and increasingly frequent convulsions. Death occurred a short time after craniotomy and unsuccessful attempt at extirpation of the tumor.

### *Pinealoma*

Only 1 instance, also, of this rare variety of glioma was encountered. It occurred in a 14 year old boy as a large soft mass, straddling the midline and extending into the basal ganglia on either side. Histologically the tissue was composed of masses of closely

packed, epithelial-like cells containing many mitoses. These cell masses were separated into small lobules and supported by trabeculae of delicate fibrous tissue thickly infiltrated with lymphoid cells. All sections were uniform in appearance and corresponded closely to the classical description of a rapidly growing pinealoma.

No clinical story was available but the patient bore the evidence of the difficulty of clinical localization of the tumor in the form of scars of old parietal, and old and recent cerebellar craniotomies.

### *Spongioblastoma Polare*

Based on histological appearance, 8 tumors of this series were classified as polar spongioblastomas. This number was a considerably greater proportion — more than double in fact — than appears in Cushing's<sup>1</sup> collection. No explanation was apparent for this discrepancy.

Five of the tumors were found in the brain stem, from the olives to the optic chiasm, and in the cerebellum of patients from 6 to 38 years of age. Two occurred in the cerebrum of patients each 54 years of age. One, a small nodule hardly to be dignified by the name of tumor, appeared as a firm mass 1 cm. in diameter attached to the floor of the left lateral ventricle of a 45 year old man who died of delirium tremens. Six patients were males and 2 females. The presence of tumor was suspected clinically in all save the last patient, and extirpation was attempted in 4.

These spongioblastomas were quite uniform in histological structure. They were composed almost entirely of spindle cells arranged in fascicles of varying compactness and resembling in appearance those of a fibrosarcoma. Blood vessels were scanty and the tumor cells exhibited no special orientation toward them. Mitotic activity varied considerably from case to case. Invasive growth could be demonstrated in about half, and 1 tumor had spread locally into the meninges. In spite of the usual description given of this variety of glioma, several microscopically typical tumors contained considerable quantities of glia fibrils, apparently formed by the tumor cells. This active production of glia fibrils, taken in conjunction with the occurrence of a small tumor of typical structure attached to the ependyma, suggested that some, at least, of these so-called spongioblastomas may actually have been astrocytomas of the subependymal piloid type described by Roussy and Oberling.<sup>7</sup>

*Ganglioneuroma and Neuroepithelioma*

No examples of these extremely rare varieties of tumor were encountered in this series of autopsies.

*Unclassified Gliomas*

There remained in this series 18 tumors, mostly from the older records, which were undoubtedly gliomas but which have not been included in the preceding classification because neither tissue nor slides were available for study. However, some tentative grouping, based on the recorded descriptions, can be suggested. Eight of them were bulky, often extremely necrotic tumors in the cerebral hemispheres of adults, middle aged and older. This, taken with the recorded microscopic descriptions, would place the growths most probably in the group of the glioblastoma multiforme. One tumor, a cystic mass in the cerebellum of a young adult, was very likely a fibrous astrocytoma. The information now available on the remaining 9 cases did not permit of their classification into the subdivisions of the glioma group.

*Discussion of the Glioma Group*

The most interesting feature of the glioma group as a whole was that in spite of minor internal differences it formed in this post-mortem series almost identically the same proportion of all tumors of the nervous system as in Cushing's large surgical collection. Within the group, with the exception of the spongioblastoma, the rarer types appeared to occur in about the same relative numbers in the two series. Of the 3 common varieties of gliomas, there was in this postmortem material a significantly larger proportion of the more atypical and malignant tumors. Finally, with the classification determined to a great extent by histological structure, there seemed to be a relatively larger number of tumors of the various cellular types found in the more unusual anatomical situations in the brain, and in patients outside of the usual age groups.

## PITUITARY ADENOMA

Six cases of adenoma of the hypophysis were collected from the records. One tumor was a pure chromophobe adenoma, 4 were of the chromophile (eosinophile) type, and 1 was mixed, chiefly chromophobe.

The single chromophobe adenoma was found in a male 28 years old. He entered the hospital because of failing vision and signs of increasing intracranial pressure of 2 years duration, and died following an unsuccessful attempt at transfrontal removal of the tumor.

The 4 chromophile adenomas occurred in more elderly patients, 3 females and 1 male. The acromegalic habitus was described as slight to obvious in 3 subjects, and passed without notice in 1, a negro. They each died of various intercurrent diseases.

The clinical behavior of the single mixed adenoma was that of its predominant chromophobe element. The patient, a 46 year old female, sought treatment because of failing vision of 7 months duration, and died of shock following attempted transfrontal extirpation of the tumor.

The incidence of hypophyseal adenomas in this collection was rather less than half that shown in the sparse postmortem figures quoted by Ewing.<sup>5</sup> Their proportion of the total intracranial tumors was only one-sixth as great as in Cushing's<sup>1</sup> series. It is further interesting to note that the ratio of chromophile to chromophobe adenomas in the two series was almost exactly reversed. The chromophobe adenoma, growing more vigorously, tends early to produce urgent symptoms of pressure and failing vision, and hence bulks large in neurosurgical work. The leisurely and insidious chromophile adenoma, in many cases, may never rise symptomatically to the surgical level but remains part of the medical experience of a general hospital. The true general incidence of the two chief varieties of hypophyseal adenoma must therefore lie somewhere between the two extremes indicated by surgical and postmortem statistics.

### SHEATH TUMORS

Tumors arising from the sheaths of the nervous system formed in this postmortem collection a relatively smaller group than in surgical figures.

#### *Meningioma*

Eighteen patients having meningiomas came to autopsy. Their ages varied from 37 to 84 years, with the peak of incidence in the fifth decade. Females strikingly outnumbered males, the ratio being 5 to 1. No particular site of predilection was apparent in the distribution of the tumors within the skull. It was noteworthy, how-

ever, that the growths that produced clinical symptoms were almost all located along the brain stem, and in the middle and posterior cranial fossae, where their presence produced either localizing pressure signs or obstructive hydrocephalus. Eight tumors had apparently been silent during life. Eight others probably produced symptoms, and in 3 instances the patients had been subjected to craniotomy with unsuccessful attempt at localization and removal of the tumor. Record was lacking on 2 patients and their history could not accurately be conjectured from the anatomical findings.

The tumors varied from 1 to 8 cm. in largest dimension. Microscopically they formed a fairly homogeneous group, differing only moderately in the amount of collagen they contained. A few, however, tended to be predominantly of spindle cell type, with rare giant cells and sparse mitoses.

Included in this group of meningiomas were the only 2 cases of multiple primary intracranial tumors encountered in this study. One patient, a female of 63 years, had a small meningioma embedded in the left frontal lobe and a large glioblastoma multiforme, the immediate cause of death, in the right temporal lobe. The other, a female of 83 years, had 2 small typical meningiomas, 1 in the left frontal and 1 in the right Rolandic region. Just posterior to the latter, deeply embedded in the parietal cortex, was a 3rd and larger mass. This was a rapidly growing fibroblastic tumor arising apparently from the pia-arachnoid and considered to be an atypical and rapidly growing meningioma.

#### *Acoustic Neuroma*

Only 4 instances of tumors of the acoustic nerve were found. The much greater relative number appearing in surgical studies would seem to be another instance of a tumor of slow and characteristic clinical course tending to accumulate in larger proportion at a surgical clinic.

The 4 tumors were evenly distributed between the sexes, and 2 were in the fourth and 2 in the sixth decade of life. Two were small but histologically typical growths less than 2 cm. in diameter found incidentally in patients dead of other causes. They had apparently been quite silent clinically. The other 2 tumors were clinically diagnosed and both patients died soon after suboccipital craniotomy and unsuccessful attempt at extirpation.

## METASTATIC TUMORS

In no other group of tumors of the nervous system do surgical and postmortem statistics differ so widely as they do in the case of metastatic tumors. Neurosurgical figures give no idea of their relative frequency because, as Cushing<sup>1</sup> points out, patients with known metastatic tumors are rarely admitted to a neurosurgical clinic since so little can be done to help them.

Twenty-nine instances of intracranial metastases were encountered. They constituted 15.4 per cent of all tumors of the nervous system. On the other hand, 3.2 per cent of all cases of malignant disease were proved to have intracranial metastases, and this figure no doubt fell short of the real total as the head could not be examined in more than half of the total autopsies. This incidence is rather less than the average of about 5 per cent with brain involvement given by Willis<sup>8</sup> in his exhaustive study of the general problem of metastasis.

The frequency with which different types of primary tumors in this series gave metastases to the nervous system is exhibited in Table II.

TABLE II

Primary tumor	No. with brain metastases	Solitary	Multiple
Primary carcinoma of lung ..	8	2	6
Malignant melanoma .....	5	—	5
Carcinoma of the breast ....	4	1	3
Carcinoma of the colon .....	2	1	1
Carcinoma of the kidney ....	2	1	1
Chorionepithelioma .....	2	—	2
Carcinoma of the prostate ..	1	1	—
Carcinoma of the adrenal....	1	—	1
Hemangioendotheliosarcoma	1	—	1
Lymphatic leukemia .....	1	—	1
Ewing's tumor .....	1	1	—
Neuroblastoma.....	1	—	1
Total .....	29	7	22

Primary carcinoma of the lung was in this series the most frequent single source of metastases to the brain. Conversely, a large proportion of lung carcinomas gave rise to intracranial deposits. In a study of lung carcinoma based on this same series of autopsies, Olson<sup>9</sup> found brain metastases in 36.3 per cent of the cases in which the head was examined. Aside from its frequency, the practical necessity of considering lung carcinoma in differential diagnosis is enhanced by

the insidious course of a number of the cases. Not uncommonly the pulmonary symptoms may be trivial or absent and the patient may seek treatment for the signs and symptoms of brain tumor. Because their neurological symptoms completely overshadowed those of the primary pulmonary lesion, 3 of the 8 patients were subjected to craniotomy on a diagnosis of probable glioma.

Brief individual notes may profitably be made of certain rare cases in the metastatic group. The 2 examples of chorionepithelioma occurred in young adults, 1 male and 1 female. In the male the primary tumor was the most active component of a testicular teratoma. The testis was removed and the patient given vigorous X-ray treatment, in spite of which death occurred about a year later from cerebral and pulmonary metastases of pure chorionepitheliomatous tissue. The female patient was admitted in coma with no reliable history obtainable and autopsy was unfortunately limited to examination of the head. In both instances death appeared to have been unexpectedly sudden and due to massive hemorrhage into one of the brain metastases.

The adrenal carcinoma occurred in an elderly negro as a solitary mass in the right adrenal, with two metastases to the right parietal lobe. Quite understandably, a diagnosis of glioma was made and one mass was surgically removed. Death occurred soon after operation and the remaining mass was found at autopsy just out of range of the operative incision. The tumor was a papillary adenocarcinoma with abundant thick mucoid secretion.

The tumor designated as hemangioendotheliosarcoma was found in a female of 63 years. Clinically and pathologically the most likely primary site appeared to have been the lungs, which were found studded with bloody tumor masses 2 to 4 cm. in diameter. A few smaller masses were present in the liver and in the mucosa of the ileum. Similar masses up to 2.5 cm. in diameter were liberally scattered throughout the brain. Microscopically the tissue was richly cellular and was composed of spindle and polygonal cells interspersed with small but numerous blood channels lined with cuboidal cells. Mitotic figures were quite plentiful throughout.

No nodular deposits were present in the case of lymphatic leukemia. Instead, the entire inner surface of the dura was covered with a delicate mat of tissue from a few millimeters to a centimeter in thickness composed of closely packed small lymphocytes supported by a



very scanty vascular stroma. There was also extensive lymphoid infiltration of the pia-arachnoid, with some prolongation into the perivascular spaces but no apparent deposits in the brain substance.

In the case of the Ewing's tumor the patient, a female of 27 years, had suffered an amputation of the right leg for the primary tumor a year previously. Death was caused by a large, primarily extradural mass in the right temporal region, compressing but not invading the underlying brain. No other masses were found, either in the brain substance or in the other viscera. The tissue was microscopically typical of the endothelial myeloma of Ewing and was said in the record to be identical with the tissue from the amputated leg.

The neuroblastoma, a typical example of the sympathetic type, was encountered in a girl of 4 years. The primary tumor apparently originated in the region of the coeliac plexus and gave rise to extensive visceral metastases. The inner surface of the dura was studded with large hemispherical masses that deeply indented but did not actually invade the underlying brain.

All of the patients in this metastatic group were adults, save the child with the neuroblastoma. A significant difference in sex incidence was not apparent except in the case of the lung carcinomas, in which group males outnumbered females 3 to 1. In this series, also, the well recognized preponderance of multiple over solitary metastases was clearly demonstrated.

No case of intradural or intramedullary metastasis in the spinal cord was encountered in these autopsies.

From the foregoing discussion it is evident that intracranial metastases occur considerably more frequently in general hospital patients than would be suggested by neurosurgical statistics. It is also probable that these postmortem figures fall short of the actual incidence, since we were not permitted to examine the head in more than half of the cases of malignant disease and thereby missed a good number of metastases, both silent and suspected. Especially is attention drawn to the relatively frequent occurrence of intracranial metastases from lung carcinoma and the not uncommon tendency for the primary tumor to remain symptomatically unobtrusive. As this tumor coincides in age incidence with the commonest of the gliomas, if futile operation is to be avoided the possibility of metastasis must be considered and thoroughly ruled out in every adult tumor suspect.

## INVASIVE TUMORS

Since their manner of gaining access to the cranial cavity differs fundamentally from that involved in true metastasis, it seemed advisable to consider the invasive tumors as a separate group. As might be expected, invasive tumors were few in number, because extensive malignant tumors of the face and pharynx were relatively scarce and the skull formed a fairly effective barrier to their spread. Four such tumors were encountered. Three were carcinomas of the pharynx and antrum, extending through the base of the skull to elevate the dura and compress, but not actually invade, the overlying brain. One was a lymphoblastoma of the reticulum cell sarcoma type in a male, 26 years of age. The growth, primarily in the cervical nodes, extended into the larynx and tongue and upward through the base of the skull into the left middle cranial fossa, where it invaded superficially into the overlying temporal cortex.

The diagnosis of such invasive tumors is usually obvious on careful examination. One of the antrum growths, however, occurred in a 14 year old girl, and because of the age of the patient and the absence of local signs, save erosion of the sphenoid, craniotomy was performed on a diagnosis of possible cranopharyngioma.

## BLOOD VESSEL TUMORS

Five examples of tumor arising from the cerebral blood vessels were found. Two of these were the cystic type of capillary hemangioma of the cerebellum described by Lindau. The patients were females aged 32 and 50 years, respectively. Death in both cases was due to obstructive hydrocephalus. The retinae in these subjects were not examined postmortem but the viscera showed no cysts or adenomas, such as have been occasionally described accompanying the angiomas of the nervous system.

The remaining 3 tumors were of the type called racemose aneurysm and consisted of tangled masses of grossly recognizable vessels. Two patients were males and 1 female. Their ages ranged from 22 to 50 years. The tumors were located, 1 each, in the left occipital and the right frontal lobe, and in the right hemisphere of the cerebellum. All appeared histologically to be of the arteriovenous variety with rather thick walled vessels and spotty calcification, but direct arterial connection was demonstrated only in the cerebellar tumor. No leiomyomatous nodules were seen among the vessels.

The patient with the cerebellar tumor died following thrombosis of the right middle cerebral artery. In the case of the occipital tumor death followed intraventricular rupture and hemorrhage. The patient with the right frontal tumor, a 22 year old male, had suffered 8 years from epilepsy. After a seizure that left him with a left hemiparesis he was admitted to the hospital and a right subtemporal decompression was performed. Death occurred a month later from softening and a small secondary hemorrhage into the ventricle. None of these patients exhibited angiomas elsewhere in the body.

### CONGENITAL TUMORS

No case of cranopharyngioma occurred in this series of autopsies.

Four cholesteatomas were encountered. Two were in female patients and 2 in males. Their ages ranged from 19 to 53 years. All the tumors were located about the base of the brain and spread irregularly to a varying extent, insinuating themselves into available spaces and sometimes compressing but never invading the overlying brain. They consisted of a rind of rather pearly luster composed of stratified squamous epithelium and enclosing crumbly masses of keratinized epithelial cells, mingled in one instance with considerable oily brown fluid.

In only 1 patient, a male 46 years of age, did a cholesteatoma appear to have caused clinical symptoms. While undergoing treatment for arthritis he suddenly developed convulsions which became almost continuous and resulted in death 2 days later. At autopsy the tumor was found as a nodular mass 3 cm. in diameter pressed deeply into the inferior surface of the right frontal lobe.

Four chordomas were found among the patients coming to autopsy. Three of these were small, non-proliferating notochordal remnants over the basisphenoid, clinically silent, and merely incidental curiosities found in the course of routine examinations. Quite a few others were probably overlooked as they were a very inconspicuous object.

One tumor, however, in a female of 35 years was a true proliferating neoplasm and caused death. It grew as a large knobby mass projecting from the clivus, compressing and rotating the brain stem, and forcing its way into the white matter at the junction of the pons and medulla. It also eroded the posterior clinoid processes and appeared beneath the mucosa of the sphenoid sinus. Microscopically

the tissue consisted of cords and masses of typical physaliphorous cells embedded in a gelatinous matrix. In many areas the tumor cells produced coarse fibrils resembling myoglia fibrils, an appearance rarely observed. Because of its interest, a separate cytological study of this tumor is now in preparation and will be published subsequently.

Regarded as a group, the congenital tumors were usually clinically silent and appeared at autopsy as incidental findings, though some of the cholesteatomas might have caused symptoms had the patients lived longer. The reason for the absence of cases of cranopharyngioma, surgically the most frequent and important tumor of this group, was not apparent.

### GRANULOMATOUS TUMORS

As was true of metastatic tumors, the postmortem incidence of the so-called granulomatous tumors was much larger than their surgical occurrence. Nineteen granulomas making up slightly more than 10 per cent of all tumors were found in this series. Sixteen were tuberculomas, and 3 gummas.

The tuberculomas were about evenly distributed between the sexes, males slightly predominating. An unusually large proportion, 6 cases, were in negro patients, apparently an example of their reputed racial susceptibility to tuberculosis. The ages of the patients varied from 7 months to 60 years, but half were in the first decade of life.

In the great majority of cases the masses were about 1 cm. in diameter. Seven were solitary and 9 multiple. Of the solitary tubercles 4 were in the cerebellum, 2 in the brain stem, and 1 in the cerebrum. When multiple they involved chiefly the cerebrum, with occasionally one or more masses in the cerebellum. Tuberculous meningitis terminated 11 of the 16 cases. Six were associated with generalized miliary tuberculosis. In about half the instances the primary disease appeared in the peribronchial or abdominal lymph nodes. The others, where a source was found, were single cases of Pott's disease, enteritis, polyserositis, pulmonary tuberculosis, tuberculous pyonephrosis, and tuberculosis of the adrenals with Addison's syndrome.

Only 2 of the patients having tuberculomas were treated surgically. Cerebellar exploration was done on a child with masses in

both cerebellar hemispheres, but no attempt was made to remove them. A parietal craniotomy was performed on a man 50 years old, on a diagnosis of probable meningioma. The longitudinal fissure was found filled with large masses of tuberculous granulation tissue plastered to either side of the falx cerebri. Both patients died of tuberculous meningitis within a brief period after operation.

Three of the granulomatous tumors were gummas. Two of the patients were males, and 1 female, all in the fourth decade. Two tumors were solitary masses, one attached to the right cribriform plate, and one embedded in the right lenticular nucleus. The 3rd patient presented two masses abutting on the meninges, symmetrically placed, one on either side at the lower end of the Rolandic fissure. In all cases the masses were 2 to 3 cm. in diameter, firm, elastic and yellowish gray. The surrounding brain tissue was soft and edematous. All showed microscopically a rather ill-defined necrotic center surrounded and invaded by a mantle of vascular connective tissue thickly infiltrated with monocytes, lymphocytes and plasma cells. The cellular infiltration was strikingly perivascular in arrangement and more or less obliterative endarteritis was present.

All 3 cases of gumma were diagnosed clinically as possible gliomas, and 1 patient was subjected to a subtemporal decompression.

#### SPINAL CORD TUMORS

Four tumors of the spinal cord were found among the patients coming to autopsy. All the subjects were males. In all cases the tumor was intramedullary in position and located in the lower cervical and upper thoracic segments. In 3 patients, aged 32, 35, and 42 years, respectively, the tumors were typical slowly growing ependymomas. The 4th tumor, histologically a medulloblastoma, was found in a child of 6 who incidentally presented in addition congenital absence of the left forearm.

#### UNCLASSIFIED TUMORS

There remain for brief consideration 10 tumors that could not be placed satisfactorily in any of the preceding groups. Three of these tumors were probably small, clinically silent meningiomas, but no slides, tissue or microscopic description were available. Four others on which material and description were lacking were 1 each of a

small pineal tumor, a pituitary tumor, a probable cystic glioma obstructing the third ventricle, and a possible gumma. One tumor was originally regarded as a glioma, but autopsy was restricted to the head and the poorly preserved tissue now available resembled more some type of metastatic carcinoma.

Adequate microscopic preparations were available on the 2 remaining tumors. One, in a female patient 42 years old, was a moderately rapidly growing typical fibrosarcoma arising from the dura overlying the right temporoparietal region. The mass pressed into but did not actually invade the adjacent brain. The other tumor, found in a boy of 16, was a small cyst 13 mm. in diameter in the choroid plexus of the third ventricle. It was so situated as to act as a ball valve, occluding the aqueduct and producing a fatal hydrocephalus. Microscopically the cyst was filled with thin serous fluid. The delicate fibrous wall was infiltrated with lymphocytes and lined by one to several layers of columnar epithelium, in part ciliated.

#### SUMMARY AND CONCLUSIONS

1. The tumors found in the central nervous system in 10,592 autopsies performed at the Boston City Hospital over a period of 39 years have been collected and arranged according to the classification of Bailey and Cushing.

2. Regarding as clinically malignant all tumors of the central nervous system, they constituted 16.8 per cent of all malignant disease encountered in this series of autopsies.

3. The proportion of gliomas in this postmortem series was practically identical with that in Cushing's collection, but within the glioma group there was a significantly greater relative number of more rapidly growing malignant varieties.

4. Pituitary adenomas were relatively scarce in routine post-mortem material, and in contrast to surgical experience the chromophile type greatly predominated.

5. The proportion of metastatic and granulomatous tumors was very high, due presumably to the absence of surgical selection of cases.

6. No significant variation in the incidence from year to year of tumors of the central nervous system could be discerned in this series.

NOTE:— In conclusion I wish to express my thanks to Dr. F. B. Mallory, and to Dr. Frederic Parker, Jr., for permission to use the accumulated material of the Institute, and for encouragement and advice in pursuing this study.

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# ULTRACENTRIFUGATION OF INTRANUCLEAR INCLUSIONS IN THE SUBMAXILLARY GLANDS OF GUINEA PIGS AND GROUND MOLES\*

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Intranuclear inclusion bodies, associated with certain virus diseases, have been considered in detail in numerous cytological investigations. Whether the inclusion bodies are derived from the virus directly or represent some product of nuclear origin is a paramount question. It is believed that information can be obtained on this subject if more is known of the physical properties of these bodies, and with this objective the relative specific gravity of different intranuclear inclusions has been determined and compared with similar physical attributes of normal nuclear elements.

The ultracentrifuge designed by Beams, Weed and Pickels,<sup>1</sup> and Beams and Pickels,<sup>2</sup> has provided a new method of approach. Lucas and Herrmann<sup>3</sup> used the instrument to centrifuge rabbit cornea infected with herpes virus, some results of which are shown in Figures 8 and 9. The uncentrifuged infected corneal cell (Fig. 9) shows the inclusion body of herpes in the center of the nucleus. The body is surrounded by a halo of nucleoplasm and the chromatin is marginated against the nuclear membrane. Centrifugation brings all the basophilic staining chromatin and eosin staining oxychromatin† to the centrifugal pole (Fig. 8). The nucleoplasm forms a layer on top of the chromatin, and the inclusion body, being lighter than any

\* Aided by grants from the Rockefeller Foundation to Washington University for research in science and in virus diseases.

† The term oxychromatin is used here to designate the material identified in a normal nucleus by its greater affinity for acid than for basic dyes. Wilson<sup>4</sup> has reviewed the implications given to the term chromatin by various authors and the relation of oxychromatin to basophilic chromatin. He concludes (p. 90), "... it is preferable to retain the older term 'chromatin' provided we apply it to the whole stainable substance of the nucleus, whether basophilic or oxyphilic, and clearly recognize that basichromatin and oxychromatin are but passing phases, more or less marked and enduring, of one fundamental substance." The identity of the two substances is confirmed in at least one respect by the similarity of their specific gravities. Lucas and Herrmann<sup>3</sup> found that the two substances were intimately intermingled at the centrifugal pole; that there was no tendency for them to stratify into two layers. Luyet and Ernst,<sup>5</sup> who centrifuged the nuclei of plant cells, show the same result in all their figures from 2 to 23, but for some reason conclude that the basophilic chromatin is heavier.

Received for publication March 3, 1936.



other substance in the nucleus, is concentrated at the centripetal pole.

It might be expected that centrifugation of submaxillary gland infected with the submaxillary virus would separate the inclusion body from chromatin and nucleoplasm in the same manner but the latter proves to respond quite differently.

### METHOD

Glands from adult guinea pigs were whirled for an hour in the ultracentrifuge at a force of about a half million times gravity. The tissues were afterward fixed in Zenker's fluid with acetic acid. Control tissue from the submaxillary glands of the same guinea pig were divided into two portions. One portion was fixed immediately after its removal from the animal; the second portion was kept in a dish of physiological saline during the hour that centrifugation was in progress and then fixed at the same time as the centrifuged tissue. The purpose of two controls was to determine whether or not autolytic changes had altered the visible structure of the cell. It was noted by Lefevre and Curtis,<sup>6</sup> for example, that in the marsupium of some species of fresh-water mussels, eggs, which happened not to be fertilized, became swollen and, due to gravity, their cytosomal materials became stratified into three layers. In the present instance, however, no marked differences were visible in the control tissues fixed an hour apart and it may be concluded that the results obtained by centrifugation are not complicated by significant autolytic effects.

### OBSERVATIONS

A normal, uninfected and uncentrifuged duct cell of the submaxillary gland of a guinea pig is shown in Figure 7. It was taken from control tissue which was fixed an hour after its removal from the body. The end of the cell adjacent to the lumen of the duct is placed toward the bottom of the plate. Figure 4 shows the effect of centrifugation on a similar cell and it is similarly oriented on the plate. The cytoplasm is little, if any, changed from its normal condition and the position of the nucleus in the cytosome remains the same with the centrifugal force employed, but the chromatin within the nucleus is concentrated at the centrifugal pole. The chromatin originally adherent to the nuclear membrane is as readily thrown down as the chromatin lying in the central part of the nucleus, which suggests

that chromatin applied to the nuclear membrane is not held there by any particular quality of adhesion. Mention will again be made of this when the behavior of chromatin in inclusion-bearing cells is discussed. The basophilic chromatin granules which are thrown to the centrifugal pole lie suspended in a menstuum of oxychromatin. Sufficient centrifugal force has not yet been attained to determine the important point whether there yet may exist a difference in the relative specific gravity of these two constituents of the normal nucleus or not. One difference between the two chromatin substances, however, seems evident, namely, that the acid staining chromatin is more adherent to the nuclear membrane than the basophilic chromatin and due to its high tenacity strands of it stretch across the nucleus parallel to the axis of centrifugal force. The same intermingling of the chromatin substances is evident in the corneal cell infected with herpes (Fig. 8), and in this case it seems evident from the work of Lucas and Herrmann<sup>3</sup> that oxychromatin is not a constituent of the inclusion body.

The considerable resistance offered by mammalian tissue cells stands in contrast to the more fluid condition found in certain plant cells. Luyet and Ernst<sup>5</sup> find in plant cells that a centrifugal force of 30,000 times gravity is sufficient to pull the nucleus into two pieces: one portion, containing the nucleoplasm, moves centripetally until it comes to lie in the upper part of the stratified cytosomal layers; the other portion, containing the chromatins, moves through the denser strata of the cytosome and rests against the cell membrane on the centrifugal side. The centrifugal force used by Luyet and Ernst on plant tissues is about one-sixteenth as great as used in the present work on mammalian cells. Beams and King obtained about the same concentration of chromatins in spinal ganglion<sup>7</sup> and in uterine gland cell nuclei<sup>8</sup> at forces of 400,000 times gravity as was obtained in the nuclei of submaxillary gland cells.

Many descriptions and illustrations have been published of submaxillary gland duct cells which contain the intranuclear inclusions characteristic of the action of the virus in this tissue (Wilson and DuBois,<sup>9</sup> Cole and Kuttner,<sup>10</sup> Kuttner,<sup>11</sup> Cowdry,<sup>12</sup> Scott,<sup>13,14</sup> Scott and Pruett,<sup>15</sup> Cowdry and Kitchen,<sup>16</sup> Pearson,<sup>17</sup> Andrewes,<sup>18</sup> Farber and Wolbach,<sup>19</sup> Thompson,<sup>20</sup> Kuttner and Wang,<sup>21</sup> Rector and Rector,<sup>22</sup> and Cowdry and Scott<sup>23</sup>). The cell and its nucleus are greatly hypertrophied. The inclusion body varies from spherical to elongate

and the chromatin is symmetrically distributed over its surface. Thompson,<sup>20</sup> who described intranuclear inclusions in the duct cells of the submaxillary glands of the rat, is the only investigator thus far to observe a striking asymmetrical distribution of chromatin. She states that there is a crescent of nuclear material on one side of the inclusion body, but does not mention, however, whether this asymmetry still exists when a cell is examined through the series of sections that cut it. Figure 5, an uncentrifuged duct cell containing an intranuclear inclusion, has a shape and distribution of chromatin not altogether typical but is presented to compare with Figure 6. It is typical, however, to the extent that it shows a halo around the inclusion body and that little and probably no basophilic chromatin is margined against the nuclear membrane. Whether or not margination of chromatin is a characteristic of these cells is discussed below.

Centrifugation throws both the eosin staining inclusion body and adherent chromatin to the centrifugal pole (Figs. 3 and 6); the nuclear membrane at that pole is bulged into the cytoplasm by the mass, which indicates that the specific gravity of the mass is greater than that of the cytoplasm and is suggestive of the effects obtained by Luyet and Ernst<sup>5</sup> in normal plant cells. The nucleoplasm is pressed to the centripetal pole. Inclusion and chromatin must have almost identical specific gravities or else the adhesion between the two is so great that the centrifugal force employed was insufficient to separate them. Search was made for inclusions around which the chromatin was unequally distributed, as described by Thompson, in the hope that the heavier element would be indicated by its rotation to the centrifugal pole. No such inclusion was found, although from inspection of a single section the chromatin frequently appears asymmetrically distributed, but when the nucleus is traced through a series of sections the chromatin proves to be equally distributed. An example of apparent asymmetry is given in Figure 3, in which the chromatin seems to be on the centripetal side of the inclusion body, but in adjacent sections there is an equal amount laterally and on the centrifugal side. Sometimes the chromatin in the uncentrifuged cell is massed at the ends of an elongated inclusion body (Fig. 5). Centrifugation of such a cell (Fig. 6) throws the mass to one end of the nucleus and in so doing it may apparently be shortened in length, but the chromatin masses adherent to it retain their original orien-

tation. The viscous oxychromatin, seen in the uncentrifuged cell (Fig. 5) and described by Thompson as a network uniting the inclusion body and chromatin to the nuclear membrane, is stretched out by centrifugation. It still retains many points of attachment to the nuclear membrane and to the inclusion body with its adherent chromatin. Evidence that the oxychromatin is concentrated at the centrifugal pole is not as definite in the infected cell of the guinea pig as it is in the normal duct cell (Fig. 4), or in the infected cell of the ground mole (Fig. 2), perhaps, because it is not as abundant in proportion to the volume of the nucleus as it is in less hypertrophied or normal cells. Cowdry and Kitchen<sup>16</sup> noted radiating strands of acid staining material extending from the inclusion bodies of yellow fever to the nuclear membrane and if the inclusion is suspended by such a viscous medium it may account for the fact that Brownian movement of the granules does not occur when living cells are examined *in vitro*. On the other hand, a cytoplasmic inclusion, vaccinia, in the fragile cells of the chorio-allantoic membrane of the chick when placed in distilled water will under these conditions show rapid Brownian movement of the inclusion granules (Goodpasture, Woodruff and Buddingh<sup>24</sup>).

There is an important difference between the behavior of chromatin in nuclei of the cornea infected with herpes and that of nuclei of duct cells infected with submaxillary gland virus. The chromatin marginates in the former which suggests that some form of antagonism exists between it and the inclusion body. There is no margination of basophilic chromatin in the latter; all of it adheres to the inclusion body. Examination of uncentrifuged cells indicates that this is the case but centrifugation emphasizes the fact more clearly that no chromatin is present which is not attached to the inclusion body. Were some of the chromatin applied to the nuclear membrane, as it is in the normal cell nucleus (Fig. 7), or as it is in herpes-infected cells (Fig. 9), it is assumed that it would have been concentrated at the centrifugal pole in the inclusion-bearing cells of the submaxillary gland. The only marginated material in the infected duct cell displaced centrifugally independent of the inclusion body is a small quantity of acidophilic granular substance, oxychromatin.

Formed elements of the cytosome are not thrown down as readily as are nuclear structures and, with the centrifugal force employed, occur only in some of the infected cells. There seems to be no notable

shifting of cytoplasmic structures in the normal cell, nor does the nucleus as a whole change its position in relation to the cytosome. The cytosomic inclusion bodies of infected cells (Pearson<sup>17</sup>) are moved somewhat in a centrifugal direction (Figs. 3 and 6). The individuality of these basophilic bodies and their centrifugal displacement is shown more clearly in Giemsa stained preparations than in those used for illustration which have been stained with hematoxylin and eosin. The non-staining ground substance is somewhat concentrated at the centripetal pole.

Intranuclear inclusions in the submaxillary glands of ground moles were discovered by Rector and Rector.<sup>22</sup> They found the inclusions are similar in most respects to the submaxillary gland inclusions of guinea pigs; the principal difference is the basophilic character of the inclusion body in the mole.

Submaxillary glands of the mole were centrifuged in the same manner as the guinea pig tissue. The results are the same (Fig. 2): the inclusion body is thrown to the centrifugal pole and the chromatin does not separate from the inclusion body. Rector and Rector report that marginated chromatin is lacking in the inclusion-bearing cells (see also Fig. 1). The absence of marginated chromatin is confirmed when these cells are centrifuged. Some oxychromatin is brought to the centrifugal pole, which indicates that the affinities of oxychromatin and basichromatin for the inclusion body are not the same, thus pointing to a second physical difference between the two types of chromatin; the first, already mentioned, is the unequal tenacious nature of the two substances.

## DISCUSSION

The centrifugation experiments have shown that in the guinea pig chromatin of an infected submaxillary gland cell is not marginated. Cole and Kuttner<sup>10</sup> noted that the chromatin granules are variously distributed in the halo separating the inclusion body from the nuclear membrane. Some of these granules lie adjacent to the membrane but retain their irregular or spherical form and are not described as marginated in the same sense as is usually applied to the phenomenon of margination in cells infected with herpes or yellow fever. Farber and Wolbach<sup>19</sup> described the distribution of chromatin in inclusion-bearing cells of the human salivary gland in about the same terms as Cole and Kuttner, namely, that the chromatin is

concentrically arranged around the inclusion body as dense staining masses, sometimes lying in the clear zone around the inclusion and sometimes adjacent to the nuclear membrane. A similar situation exists in the infected salivary gland of the Chinese hamster, *Cricetus griseus*, M. Edw., and in the mouse (Kuttner and Wang<sup>21</sup>). Thompson, studying the rat, and Rector and Rector, the mole, state that there is little or no tendency for chromatin to marginate in infected salivary gland cells of these animals. The condition described by some of the investigators who mention that some chromatin is found adjacent to the nuclear membrane may be illustrated by the isolated clump of chromatin in the lower left hand side of Figure 4. It appears to lie adjacent to the nuclear membrane, yet its adhesion to the inclusion body is evident when the cell is centrifuged. Were the chromatin not adherent to the inclusion body, it would presumably form a stratum at some level, either below or above the inclusion body. Such stratification is clearly exemplified in herpes-infected cells where margination is a definite characteristic. In the guinea pig the chromatin adheres to the surface of the inclusion body but in the mole the attraction between the two substances has gone farther and the chromatin seems to be dissolved in the inclusion body; if not actually dissolved, certainly one can safely say they are thoroughly intermingled, which indicates a compatibility between the two substances not found in viruses which produce margination. This mingling of basophilic chromatin is probably responsible for the varying degrees of basophilia described in the inclusions of submaxillary glands of various animals and man and may well be a measure of the compatibility of the two substances. That the degree of basophilia is due to different amounts of chromatin is corroborated by the faint positive reaction given by the Feulgen thymonucleic acid test for chromatin on the inclusions of guinea pigs (Cowdry<sup>12</sup>) and the more positive test obtained by Rector and Rector<sup>22</sup> for the inclusion bodies of the ground mole.

The specificity of viruses for particular animals and certain tissues is a well known distinctive characteristic of this group of pathogenic organisms. As a result of these experiments, and also from a survey of the literature, a correlation between the high specificity of submaxillary gland viruses in different animals and their close compatibility with the nuclear substance of the cells in which they occur is suggested. Demonstration of specificity

has been offered repeatedly: for example, Cole and Kuttner,<sup>10</sup> and Kuttner<sup>11</sup> were unable to transmit the submaxillary virus of the guinea pig to young rabbits, rats, kittens, chickens, pigeons, dogs or *Macacus rhesus* monkeys. Kuttner and Wang<sup>21</sup> were unable to transmit the submaxillary gland virus of the Chinese hamster to young guinea pigs or to rabbits. The same negative results were obtained in attempts to transmit the human submaxillary gland virus to young guinea pigs, hamsters, mice, rats, rabbits and monkeys. Likewise attempted passage from mice to young guinea pigs failed. When passage was effected from wild rats to laboratory rats only a mild reaction was obtained, demonstrating a specificity so great that animals as closely related as the two varieties of rats give different responses to the virus. Rector and Rector<sup>22</sup> were unable to transmit the virus from ground moles to young guinea pigs, rabbits, white mice or rats.

When the cytology of the inclusions, as found in the salivary gland duct cells of various animals, is considered it is noted that they have in common an absence of margination. It has already been noted that no one who has examined these inclusions closely describes or pictures margination of the type found in herpes and yellow fever. Centrifugation and staining techniques have demonstrated that the chromatin is associated with the inclusion bodies of submaxillary gland infections to varying degrees of intimacy.

If the suggestion is valid that the affinities and specificity of a virus are indicated by and correlated with the degree of compatibility between the inclusion body and the basophilic chromatin, then the converse should exist — namely, that viruses capable of transmission into a variety of hosts and tissues should cause margination of chromatin. Herpes is a classical example of a cosmopolitan virus producing intranuclear inclusions. It produces these inclusions in man and in many animals, such as the rabbit, guinea pig, rat, mouse, *Cebus* monkey and the chick embryo; and in many tissues, such as conjunctiva, cornea, retina, buccal mucosa, skin, trachea, liver, adrenal, ovary, testis, several different cells of the central and sympathetic nervous system,<sup>25, 26</sup> and fibroblasts in tissue culture.<sup>27</sup> The degree of pathogenicity may vary in different tissues and in different animals, but the occurrence of margination in inclusion-bearing cells always takes place. It is especially striking in those cells for which the virus does not have a natural affinity. That such is the case

is demonstrated in the colored illustration by Goodpasture and Teague<sup>25</sup> showing infected cells of the tracheal epithelium. The tracheal epithelium is, in a sense, a foreign host for the virus. The cytological picture expresses this relation in that margination, indicative of a chromatin-inclusion antagonism, is very pronounced. This is best shown in the younger stages where the inclusion body is small and lies in the center of a great halo created by the vigorous repulsion of the chromatin against the nuclear membrane. Goodpasture and Teague have also provided a standard of comparison, namely, a colored reproduction showing the effect of herpes on nerve cell nuclei, and in a succeeding article<sup>28</sup> have demonstrated the particular affinity which this virus (strain M) has for the nervous system. Since the nervous system is the "normal" habitat of this virus, just as the submaxillary gland is the "normal" habitat of the submaxillary virus, it is to be expected that the compatibility between chromatin and inclusion body would be greater than was shown in the infected epithelial cells of the trachea. That such is the case is evident when their Figure 2 is compared with Figure 1<sup>25</sup>: in the nerve cell nuclei, chromatin and inclusion granules readily mingle. The inclusion is not concentrated in a compact mass in the center nor is the chromatin severely margined.

Thus far two extremes have been considered: (1) the submaxillary gland virus group having high specificity and no margination, and (2) herpes having low specificity and pronounced margination, especially in those tissues for which it does not have a natural affinity. The specificity of many viruses lies between these two extremes and one should find intermediate stages in the degree of margination or, expressed differently, intermediate degrees of compatibility between chromatin and inclusion body. There are two viruses, yellow fever and virus III, which fall in this intermediate category about which sufficient is known cytologically to make adequate comparisons with herpes. The greater specificity of yellow fever over herpes is indicated by the few mammals aside from man which are susceptible to yellow fever, and also by the few tissues within a susceptible animal, such as the *rhesus* monkey, which will develop intranuclear inclusions. This being the case, the chromatin of a liver cell should show greater tendency toward compatibility with the inclusion body of yellow fever than of herpes. Cowdry and Kitchen<sup>16</sup> make these statements concerning the cytology of liver cells affected with these



viruses (page 246). "... it was found that the discrepancy in the staining properties is occasioned not by any recognizable difference in the reactions of the individual acidophilic particles which make up the inclusions, but by retention, in the early stages, of more unmarginated basophilic material in the yellow fever inclusions, as compared with those of herpes. . . . The separation, or cleavage, between the acidophilic and basophilic constituents of the nucleus is therefore more marked in herpes than in yellow fever."

"... when herpetic inclusions begin to form in a localized part of the nucleoplasm they are generally limited by a halo of unstainable substance in which there is no basophilic chromatin. Halos of this kind are not so easily found in yellow fever. The usual appearance in the latter disease is represented in figures 15 and 16 where no disturbance in the distribution of the basophilic material in the immediate vicinity of the developing inclusions is at first to be detected. And . . . , we may mention a distinction which is of all the easiest to make between nuclei affected by the two viruses. In the case of yellow fever the amphinucleolus generally maintains its central position in the nucleus until after the inclusions have become well developed, as is depicted in Figures 15 to 20. In herpes, on the other hand, the basophilic component is more quickly split off from the amphinucleolus, and the remaining substances become marginated on the nuclear membrane with the rest of the nuclear chromatin. For this reason central solitary nucleoli in nuclei containing mature nuclear inclusions are rare in herpes but common in yellow fever. The influence, whatever it is, which causes the margination of basophilic chromatin and the central accumulation of the acidophilic fraction sweeps through the nucleus in a more unrestrained way in herpes than in yellow fever."

Virus III likewise has a much greater animal specificity than herpes, in fact almost as great as found in submaxillary gland viruses, but does not have the same high degree of tissue specificity. In the rabbit, virus III is capable of producing intranuclear inclusions in as nearly as great a variety of tissues as herpes. Cowdry<sup>29</sup> notes that virus III produced inclusions in the following situations: "... (1) endothelial cells, (2) macrophages, (3) interstitial cells, (4) spermatogonia, (5) spermatocytes I and II (occasionally), and (6) epithelial cells of the tubuli recti, canals of the rete, ductuli efferentes and ductus epididymis." Rivers and Tillett,<sup>30</sup> Rivers and Stewart,<sup>31</sup> and

Miller, Andrewes and Swift<sup>32</sup> had previously shown that virus III will produce inclusions also in the cornea, skin, glial and nerve cells of the brain, epithelial cells of chorioid plexus, pericardium and heart muscle. Are the cytological configurations produced by virus III and herpes correspondingly proportional to the host and tissue specificity which they may possess? Cowdry<sup>29</sup> observed that nuclei containing herpetic inclusions are more hyperchromatic than the nuclei containing virus III inclusions. This interpreted on the basis of a "compatibility theory" means that the antagonism between inclusion and chromatin being greater in herpes, the chromatin is more vigorously concentrated against the nuclear membrane, thereby rendering its appearance more hyperchromatic. Additional confirmation is given of greater compatibility of chromatin for the virus III than for herpes inclusion body when the Feulgen thymonucleic acid test is applied. The observed facts presented by Cowdry are: "Whether the color of the herpetic inclusions is sufficiently marked to justify the listing of the majority of herpetic inclusions as feebly positive is doubtful.

The virus III inclusions react a little more strongly. It is rare to find any which do not exhibit just a tinge of rose pink. In the case of the most compact ones, the rose pink is replaced by a light mallow purple. But this mallow purple is still very much lighter than the color taken by the nuclear chromatin. It is not unusual to find in the testicles inoculated with virus III that some of the halos separating the inclusions from the surrounding nuclear membranes are themselves evenly colored a pale rose despite the fact that they are devoid of visible contents."

There is still a wide gap between the submaxillary virus inclusions which do not produce margination and the virus of herpes, yellow fever and virus III which do. This gap can be bridged partially, at least. When the submaxillary gland virus is inoculated intracerebrally or intraperitoneally into a guinea pig some mononuclear leukocytes show intranuclear inclusions. This is not a cell for which the virus has a natural affinity and, as might be expected, lack of adaptation is expressed by an antagonism which produces margination of chromatin. It remains to be determined by centrifugation experiments to what extent this antagonism occurs in the transplantation of the virus into a foreign cell. Such experiments are already underway. Recently Cowdry and Scott<sup>23</sup> have described an intranuclear in-

clusion in the submaxillary gland duct cells of the *Cebus fatuellus* monkey. From their illustrations and descriptions the reaction of the chromatin to the inclusion is an excellent example of a condition intermediate between the close compatibility of the substances found in the ground mole and guinea pig and the antagonism present in herpes. It is hoped that someone to whom a supply of living *Cebus* monkeys is available will determine the specificity of the virus producing the submaxillary gland inclusion.

It is realized that the whole field of intranuclear inclusions has not been surveyed but the discussion has been limited to those of which I have had more or less personal knowledge. It is acknowledged that before this suggested correlation between host or tissue specificity of the virus and chromatin-inclusion compatibility can attain weight as a working hypothesis, it is necessary that inclusions such as those of varicella, fox encephalitis,<sup>33</sup> Rift Valley fever,<sup>34</sup> Borna disease, poliomyelitis,<sup>35,36</sup> mad itch of dogs,<sup>37</sup> Brazilian virus,<sup>38</sup> and many others be considered and in some cases reexamined cytologically in different stages of development\* and subjected to centrifugation experiments. More data will have to be accumulated concerning the specificity of the virus before the validity of the suggestion will either gain credence or be unequivocally disproved.

It would seem, however, from the work done thus far that perhaps the reaction of a virus to its host tissue may ultimately find elucidation in the same fundamental biological principles that underlie the compatibility of homoplastic and heteroplastic tissue transplants and of cross-fertilization between different species and genera.

#### SUMMARY AND CONCLUSIONS

1. When submaxillary gland tissue is centrifuged, normal nuclei of duct epithelial cells are modified: both basi- and oxychromatin are concentrated at the centrifugal pole and the nucleoplasm, being lighter, is moved centripetally. There appears to be no difference in specific gravity between basi- and oxychromatin, but the latter resists separation from its attachments to the nuclear membrane and other objects in the nucleus.

\* It is obvious that comparisons and conclusions can be made more accurately if the cytological changes are observed at various stages during the formation of the inclusion body. Where margination occurs, it is known that the development of the condition is progressive; therefore a comparison of an early stage of inclusion body formation of one virus with a late stage of another virus would obviously lead to error.

2. Duct cells of the guinea pig or ground mole containing intranuclear inclusion bodies produced by their submaxillary gland viruses respond to centrifugation by displacing both basic chromatin and inclusion body to the centrifugal pole of the nucleus. It is apparent from centrifugation that the basic chromatin is strongly adherent to the inclusion body. In the ground mole the association between inclusion body and chromatin is more intimate than it is in the guinea pig.

3. A correlation is suggested between the chromatin-inclusion body relation and the specificity which a virus has for a particular host or tissue. When a virus is very selective, as it is in the submaxillary viruses, generally there is found a corresponding compatibility between inclusion body and the chromatin of the infected cell. In contrast, viruses having low specificity, such as herpes, which is cosmopolitan in infective potentialities, show low compatibility with the nuclear material. This is expressed in margination of chromatin and is indicated also by the results of centrifugation experiments.

4. The literature on viruses producing intranuclear inclusions furnishes examples showing various degrees of compatibility of the chromatin for the inclusion body and of the infective specificities of those viruses for certain hosts or tissues. These examples seem to fit into the theory suggested that the cytological picture may prove to be a measure of the infective specificity of the virus and that both have a common origin in the same biological principle.

After this manuscript went to press the publication of R. G. Green<sup>39</sup> came to my attention. His concept of the retrogressive and adaptive evolution of viruses harmonizes closely with the work reported here. Applying his conclusion, it seems quite reasonable to interpret the different cytological pictures as expressions of the degree of adaptation that has been attained by the viruses.

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## DESCRIPTION OF PLATE

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### PLATE 151

The magnification of the published figures is 2750 times. The direction of centrifugal force is indicated by arrows in Figures 2, 3, 4, 6 and 8. The following abbreviations are used: basich., basichromatin; cyto. incl., cytoplasmic inclusion; incl., intranuclear inclusion body; mar. basich., marginated basichromatin; nuc. m., nuclear membrane; nucpl., nucleoplasm; oxych., oxychromatin.

FIG. 1. A duct cell of the submaxillary gland of the ground mole which contains an intranuclear inclusion. The basichromatin is closely associated with the inclusion body.

FIG. 2. A cell similar to the one shown in Figure 1 and from the same gland, which has been centrifuged for an hour at about 500,000 times gravity.

FIG. 3. A duct cell of a guinea pig submaxillary gland which contains an intranuclear inclusion produced by the guinea pig submaxillary gland virus. The cell has been centrifuged for an hour. Basichromatin is not separated from the inclusion body by the treatment.

FIG. 4. An illustration of a normal duct cell of the submaxillary gland which shows the effect of centrifugation. Both basichromatin and oxychromatin are concentrated at the centrifugal pole. Part of the latter is still attached to the nuclear membrane.

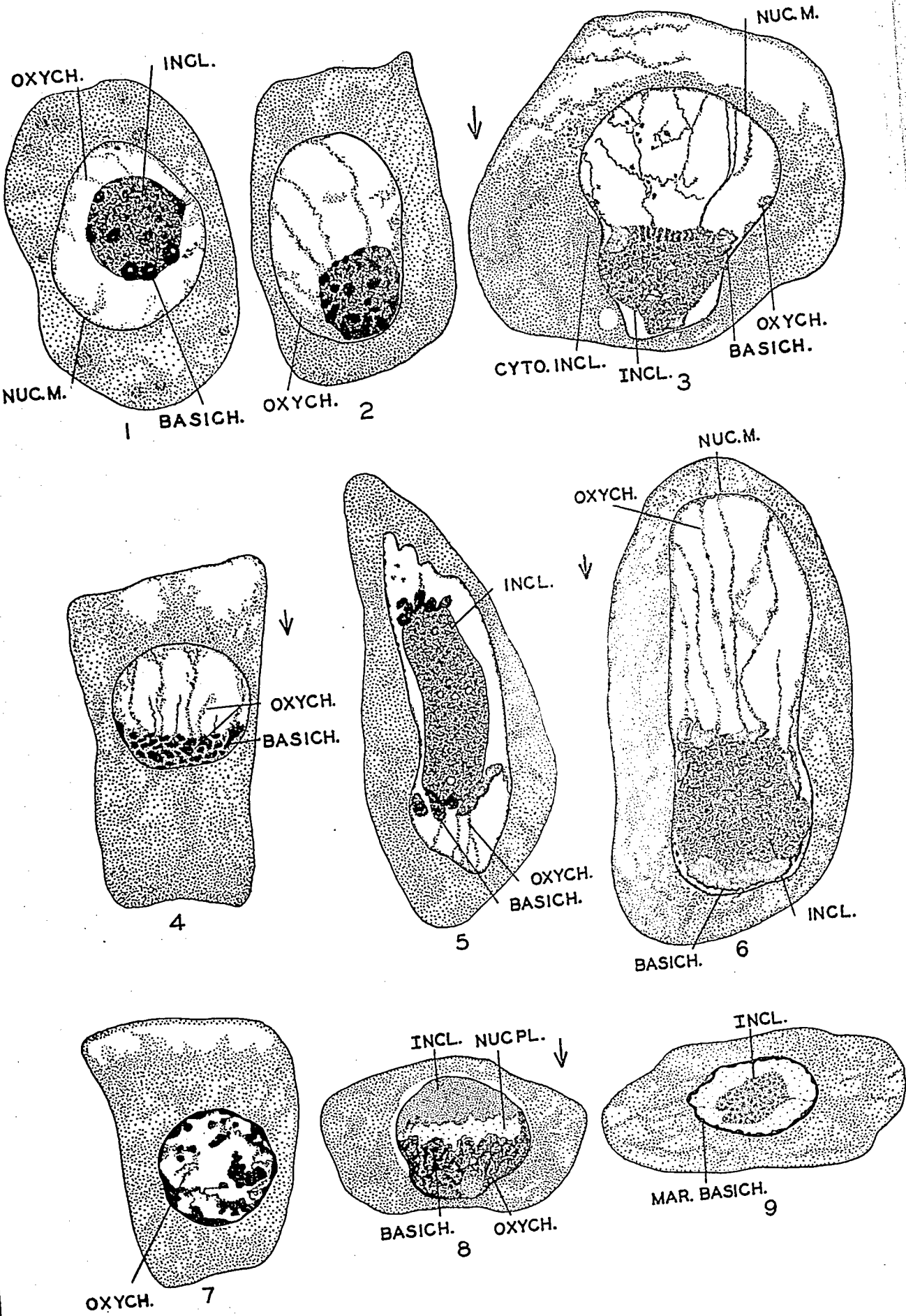
FIG. 5. An uncentrifuged infected duct cell of the submaxillary gland of the guinea pig.

FIG. 6. The effect of centrifugation on a cell similar to the one shown in Figure 5.

FIG. 7. A normal, uncentrifuged salivary duct cell shown for comparison with Figure 4 and with infected cells.

FIG. 8. A cell from the corneal epithelium of the rabbit infected with herpes virus. Centrifugation separates inclusion body, nucleoplasm and chromatin into three strata.

FIG. 9. An uncentrifuged corneal cell containing a herpes inclusion body for comparison with Figure 8.



Ultracentrifugation of Intranuclear Inclusions





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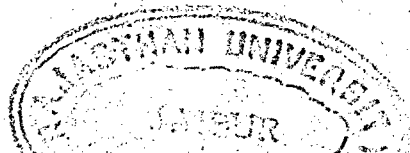
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Nevertheless, studies of the minute histological changes in the smaller blood vessels and alveolar capillaries have not been numerous. A detailed analysis of the relation of the morphological changes in the minute pulmonary vessels to structural alterations in the alveolar wall is also lacking. Thus it seems to us timely to analyze the physiological significance of the morphological alterations within the lungs in the presence of heart disease.

In a recent article zu Jeddloh<sup>3</sup> has presented a review of the past studies of pulmonary congestion and in addition has reported his own observations on changes in the alveolar walls in chronic passive congestion. He did not include a study of the vascular changes apart from those of the capillaries. Brenner<sup>4</sup> has reviewed the literature on the pathology of pulmonary vessels; hence the presentation of such data is superfluous.

Our interest in this subject was aroused by the rather unusual clinical behavior and striking postmortem findings in a case of mitral stenosis, which is reported below. The study was later extended to a large group of cases presenting various types of pulmonary parenchymatous and vascular disturbances.

#### MATERIAL AND METHODS

In addition to the case reported in detail below, we have studied 9 cases of mitral stenosis. We have also examined the lungs in cases of rheumatic heart disease of varied pathology, of arterial hypertension, congenital heart disease, cardiac asthma, bacterial endocarditis, and in 1 case of marked kyphosis with right-sided hypertrophy and heart failure.

In the material under study during the past 2 years sections were taken from the upper and lower parts of each lobe and were run through separately. The tissues were fixed in Zenker's fluid and were routinely stained with phloxine-methylene blue and the Lee-Brown modification of Mallory's aniline blue connective tissue stain. In certain instances elastic tissue and reticulum stains were also employed.

Measurements of the structural components of the alveolus were done with the aid of a micrometer. In order to correct the error due to shrinkage of tissues, the instrument was calibrated in relation to the size of the red cells contained within the lung tissue. The average

diameter of 10 to 20 red cells was accepted as indicating 7.5 microns. The number of capillaries visible in normal and in congested lungs was counted.

## RESULTS

### *I. Structure of Normal Alveolar Wall*

Before proceeding to a description of the pathological changes in the minute vessels and corresponding alveolar wall, it would seem

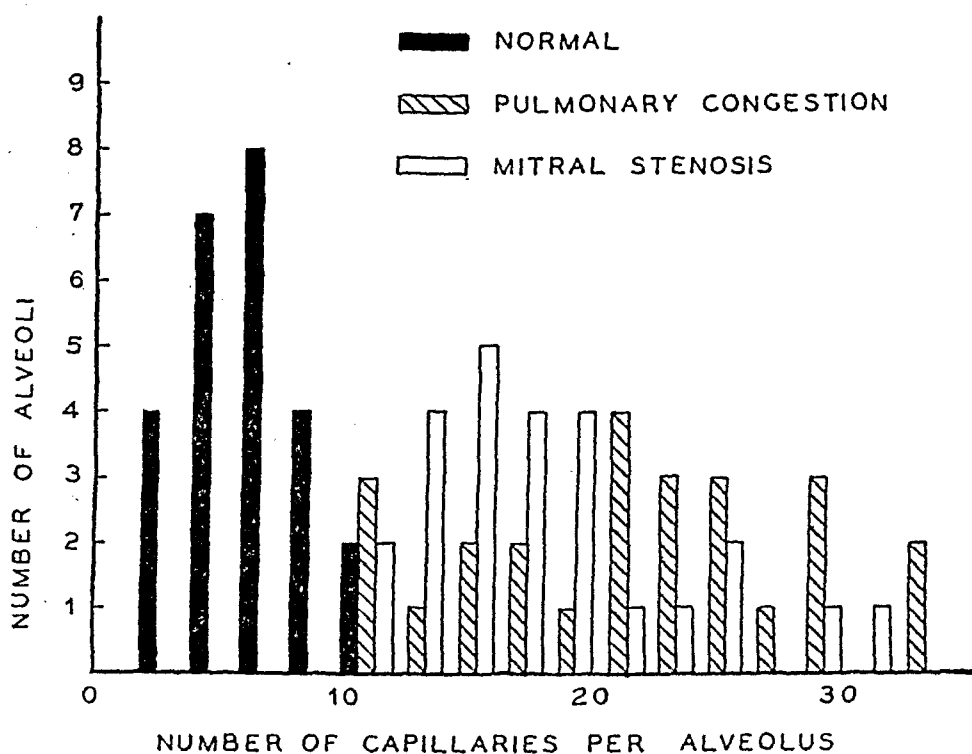


CHART I

Comparative distribution of the number of visible ("open") capillary lumens per cross-section of a single alveolus in the normal lung, in the lung with simple congestion, and in advanced mitral stenosis

wise to review briefly the normal structure and dimensions of the alveolar wall.

The alveolar wall is covered by a layer of what, in our opinion, are flattened epithelial cells. Beneath these cells is a delicate band of collagen, the so-called alveolar basement membrane. This appears as a homogeneous structure, staining light blue with the Lee-Brown stain. Often placed somewhat eccentrically in the wall and close to the alveolar surface is a capillary whose lumen is sufficiently

wide to admit with ease the passage of a single red cell. In the normal lung the average diameter of the capillary lumen is between 9 and 10 microns. In the absence of congestion one sees relatively few capillaries with a diameter of over 14 microns. Surrounding these capillaries is a layer of collagen, the capillary basement membrane. The thickness of the tissues between the alveolar space and the capillary lumen is between 1 and 2 microns, while that of

TABLE I

*Comparison of Diameters of Capillaries and of the Corresponding Alveolar Wall of the Normal Lung and the Lung in Advanced Mitral Stenosis*

NORMAL		MITRAL STENOSIS	
Capillary ( $\mu$ )	Alveolus ( $\mu$ )	Capillary ( $\mu$ )	Alveolus ( $\mu$ )
9 .....	16	20 .....	54
14 .....	17	25 .....	67
9 .....	15	16 .....	45
10 .....	14	10 .....	68
12 .....	16	26 .....	26
9 .....	12	28 .....	55
9 .....	12	20 .....	72
9 .....	14	30 .....	68
10 .....	15	12 .....	70
8 .....	12	7 .....	41

the opposite side of the capillary is between 2 and 3 microns. Ordinarily the alveolar and capillary basement membranes are so close together that they appear as one layer, whereas they are quite separate. This is demonstrated, as will be shown, in instances where there is edema of the wall or an infiltration of cells between the two membranes. In addition to the above components, there are elastic fibers, an occasional delicate bundle of collagen, and a rare fibroblast or histiocyte.

Within the transverse diameter of the alveolar wall there is but 1 capillary. Along the circumference of a cross-sectional surface of the alveolar wall we have counted from 2 to 10 capillary cross-sections, with an average of about 5. Whether these visible cross-sections represent separate capillaries or whether some are parts of the same capillary cannot be stated.

The structure of the normal alveolar wall may be diagrammatically represented, as in Figure 3. Sample measurements of the normal capillary lumen and the alveolar wall, the ratio of the diameter of the capillary to the thickness of the alveolar wall and the relation of these findings to similar measurements obtained in the case of

mitral stenosis are presented in Table I. Chart 1 shows the number of capillaries visible ("open") per cross-section of a single alveolus in a normal lung, as compared with the number visible in congested ones. The difference, which was a consistent finding in counting the capillaries of numerous alveoli, indicates that in the normal lung as compared with the diseased, as observed postmortem, a considerable proportion of the capillaries are collapsed.

## *II. Structure of Minute Vessels and of Alveolar Wall in Cases with Cardiac and Pulmonary Pathology*

### *Report of a Case*

*Clinical History:* On Dec. 29, 1933, a 33 year old Irish-American female candy worker was admitted to the Boston City Hospital with the history of "heart trouble" of  $2\frac{1}{2}$  years duration.

At the age of 10 years the patient was out of school for 6 months with a "nervous condition," probably chorea. At the age of 15 she suffered from generalized joint pain, with tenderness, swelling and redness. This condition lasted but 1 or 2 weeks. Six years before admission to the hospital she developed dyspnea on exertion, and suffered from coughing spells 5 to 6 times a year. About  $2\frac{1}{2}$  years ago a heavy tray was dropped on the patient's head. Within a few hours she developed severe cyanosis, precordial pain and orthopnea. The pain radiated to the left side of the back and was accompanied by a sensation of heat down the left arm. Ever since this episode the patient had been an invalid, confined to bed during the greater part of the day. On several occasions she had been troubled by attacks of severe dyspnea, orthopnea, palpitation and precordial pain. During the past year, on 5 occasions she experienced severe attacks of a choking sensation with obligatory orthopnea, followed by rather profuse hemoptysis. On each occasion the sudden hemorrhage was followed by the raising of dark clots and streaked sputum for 3 or 4 days. During the week before she entered the hospital the patient became intensely dyspneic and felt chilly.

The family, social and past histories contained no additional pertinent facts. There was no history of nocturia or of edema. The patient had lost some 12 kg. during her illness, weighing 42 kg. at entrance to the hospital. Her height was 157 cm.

*Physical Examination:* On admission she appeared rather poorly nourished, and had to be propped up in bed. She was quite dyspneic, the lips were intensely cyanotic and her condition seemed alarming. The head and neck were normal without evidence of venous congestion. The chest showed symmetrical and somewhat limited excursion. The lungs were resonant with the exception of the right base, which was somewhat dull. Anteriorly over the subclavicular area, moist râles, and over the axillae and posteriorly toward the base, crackling râles were heard.

The apex impulse of the heart was in the fifth space, where the maximal left border was 11 cm. A systolic thrill was felt in the fourth space on the left side of the sternum. The first cardiac sound was marked by a presystolic murmur



over the apex. There was also a diastolic murmur and a snapping second sound. A gallop type of rhythm with a third heart sound and with a markedly accentuated second sound was heard. The pulmonary second sound was also accentuated and coincidentally a "shock" was felt over the pulmonary conus. The pulses were small. The arterial pressure was 60/30 mm. Hg. on admission; later the average level was 110/60 mm.

The liver edge was felt two fingers below the costal margin. There was no subcutaneous edema. The results of the rest of the examination were irrelevant.

*Course of Illness:* During almost 3 months stay in the hospital the patient suffered from numerous attacks characterized by intense dyspnea, orthopnea and accentuated cyanosis. On such occasions the lungs filled up with bubbling moist râles and she expectorated frothy, blood-tinged fluid. The patient became livid blue and later ashy gray. On each occasion she went into circulatory collapse. The attacks were relieved with oxygen and caffeine. Between attacks she remained propped up in bed. During the first half of her last illness in the hospital the lungs cleared up fairly well between the attacks of dyspnea and pulmonary edema, but during the last 6 weeks of life coarse bubbling râles were present throughout the lung fields, particularly through the *upper part of the lungs*. Signs of fluid appeared in the right pleural cavity about 6 weeks before death, and on four occasions thereafter 1300 to 1500 cc. of amber colored fluid were removed, with temporary relief. Slight edema of the ankles appeared during the last 2 weeks. The patient expired on March 17, 1934, following an attack of dyspnea.

The temperature was essentially normal during the first 4 weeks; thereafter it rose to 101° F. at irregular intervals. The heart rate fluctuated between 80 and 120 per minute, and the respirations from 20 to 30.

*Laboratory Data:* X-ray examination of the chest (Dec. 29, 1933) revealed rheumatic deformity of the heart with congestive changes in both lungs. On Jan. 18, 1934, the diameter of the great vessels was 5 cm., the maximal transverse diameter of the heart 13.8 cm., and of the thorax 24 cm. Evidence of a small amount of fluid was present over the right base. On Jan. 24, 1934, in addition to the previous findings, localized shadows over the left axilla suggested small infarctions or pneumonia.

On Dec. 29, 1933, and on Jan. 25, 1934, the electrocardiogram revealed normal sinus rhythm. The P-R interval was 0.16 second, the Q-R-S 0.08 second. T<sub>1</sub> was flat, T<sub>2</sub> and T<sub>3</sub> inverted. The axis indicated right ventricular preponderance. On March 6, 1934, the electrocardiogram revealed auricular fibrillation.

Numerous analyses of the urine failed to reveal abnormal findings. The result of the concentration and dilution test of the urine was normal. The non-protein nitrogen of the blood was 41, 37 and 35 mg. per 100 cc. on different occasions. The Kahn test of the blood was negative. The red blood cell count varied between 4,000,000 and 5,200,000 per cubic millimeter, and the hemoglobin ranged from 69 to 84 per cent. The hematocrit reading was 42 per cent, mean corpuscular hemoglobin concentration 31.4 per cent, mean corpuscular hemoglobin 25.3 micrograms, mean corpuscular volume 80 cubic microns. The platelets were essentially normal and there was slight achromia. There was a continuous slight leukocytosis, 10,800 to 15,700 white blood cells per cubic millimeter, which on two occasions rose to 28,700 and 22,000. The polymorphonuclears varied from 63 to 86, lymphocytes 10 to 24, monocytes 4 to 10, eosinophils 1, basophils 2 per cent. Blood cultures were sterile. The chest fluid showed a specific gravity of from 1.006 to 1.012, red blood cells 2500 to 9000, white blood

cells 160 to 2200 per cubic millimeter; polymorphonuclear leukocytes predominated. The non-protein nitrogen content of the pleural fluid was 20 to 29 mg. per 100 cc.; protein 1.1 to 1.5 gm. per 100 cc.

*Clinical Diagnoses:* Rheumatic heart disease with advanced mitral stenosis; paroxysmal dyspnea (cardiac asthma) with attacks of acute pulmonary edema; chronic passive congestion and chronic edema of the lungs; right hydrothorax and congestive failure of the circulation; pulmonary infarcts.

### *Autopsy Report*

*Gross Findings:* The examination was performed 5 hours post-mortem. There was intense cyanosis of the lips and face, and marked lividity of the dependent parts. Slight pitting edema was present up to the knees. The *peritoneal cavity* contained 1000 cc. of clear amber fluid. The liver edge was 17 cm. below the xiphoid base, and 8 cm. below the costal margin on the right. The right *pleural cavity* contained 800 cc. of clear fluid; the left was obliterated by firm fibrous adhesions. The *pericardial cavity* contained 200 cc. of fluid.

The *heart* weighed 380 gm. The trabeculae of the right ventricle were moderately thickened. The endocardium of the left auricle was white and opaque and the auricular myocardium presented trabeculation. The mitral valve was rigid and sclerotic. The opening was represented by a narrow slit, 2 cm. long and 2 mm. wide (Fig. 1). The edges of the leaflets were slightly overlapping, and there was evidence of fusion of the leaflets at both edges of the fissure. The chordae tendineae were greatly thickened, shortened and calcified. The trabeculae of the left ventricle were markedly thinned. The aortic cusps showed questionable slight fusion at the commissures. The other valves were normal. The coronary arteries were also normal.

The cardiac measurements were as follows: tricuspid circumference 11 cm.; pulmonary opening 6.5 cm.; mitral slit 2 cm. long and 0.2 cm. wide; aortic opening 6 cm. The thickness of the left ventricle was 0.8 to 1.4 cm., and that of the right 0.2 to 0.5 cm. The aorta was delicate and elastic. There were traces of yellow atheromatous streaking in the lumbar region. The circumference measured 6 cm. at the ring, 3.5 cm. at the upper dorsal level, 3.4 cm. at the first lumbar vertebra, and 2.5 cm. at the bifurcation.

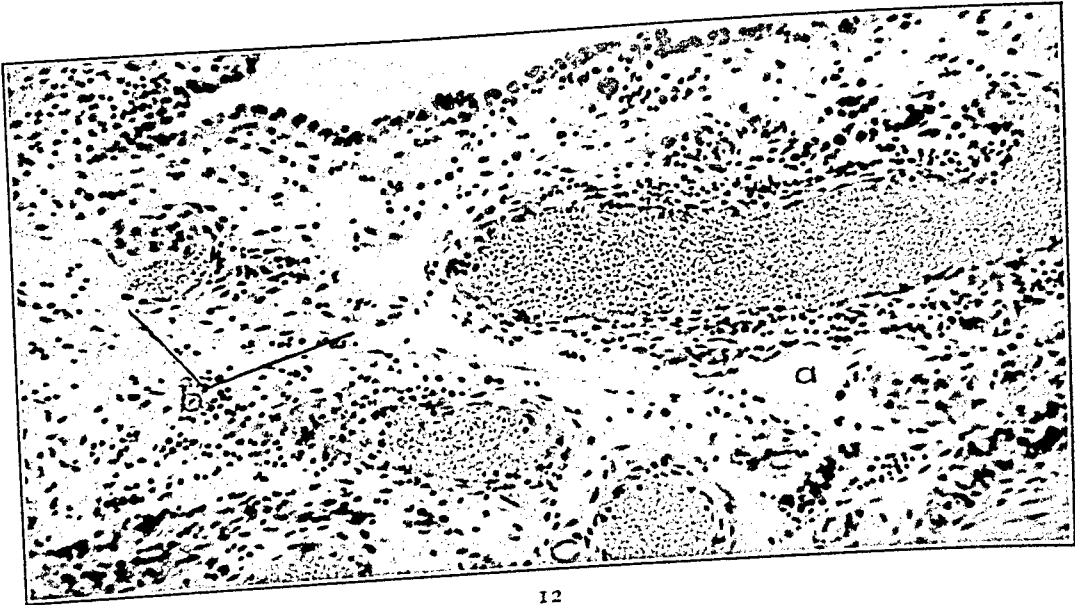
The *lungs* weighed 1350 gm.; the right 700 gm. and the left 650 gm. The pleural surface on the right was glistening, and on the left



10



11



12

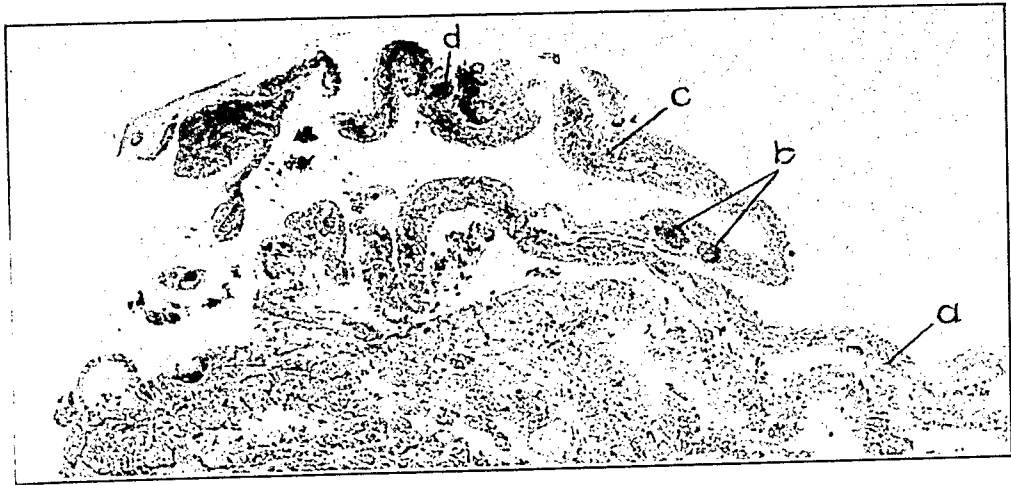
Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

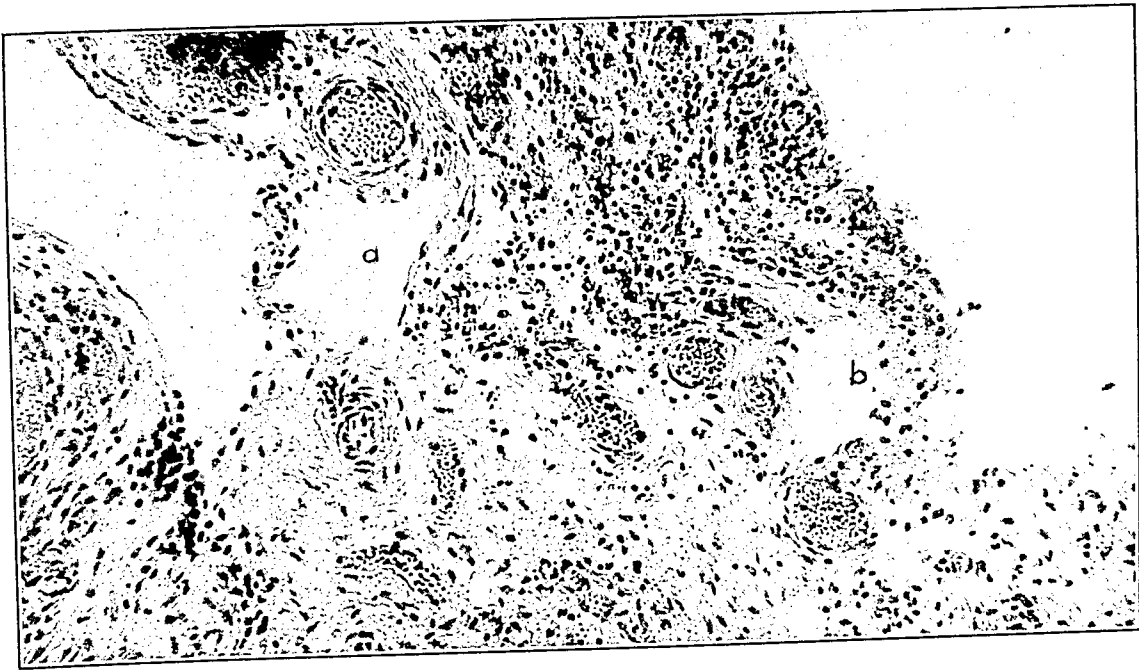
FIG. 13. Granulation tissue on the posterior surface of the right uterine cornu of the same patient (Case 1). The ovarian carcinoma was on the opposite side. Long slender strands of granulation tissue have arranged themselves in fantastically designed forms. Implantations of carcinoma, "b," "c" and "d," in various stages of development are present in these strands. One of these strands is attached to the peritoneum at "a."  $\times 10$ .

FIG. 14. Higher magnification of the attachment of one of the strands of granulation tissue, "a" in Fig. 13, to the posterior surface of the uterus. Newly formed blood vessels are present in the base of the strand and about these are two dilated spaces, "a" and "b," lined by endothelium-like cells and presenting the same histological picture as the proved lymph vessels shown in Figs. 2 and 3. Unfortunately it is impossible to establish the continuity of these structures with preëxisting lymph vessels in the underlying tissue. Lymph vessels may be present in the uterine wall beneath the base of the strand of granulation tissue but are invisible because of the occlusion of their lumina by the surrounding dense tissue. Compare with the peritoneum shown in Figs. 2 and 3, where the tissues are less dense, possibly edematous, and the lymph vessels are dilated and easily seen. We are unable to trace these possible lymph vessels "a" and "b" farther into the strand of granulation tissue in this section. However, in other nearby sections of the same strand, possible lymph vessels are found just above those shown in this illustration (see Fig. 19). These may be continuous with the vessels "a" and "b." Similar possible lymph vessels are also seen in many sections of these strands.  $\times 130$ .

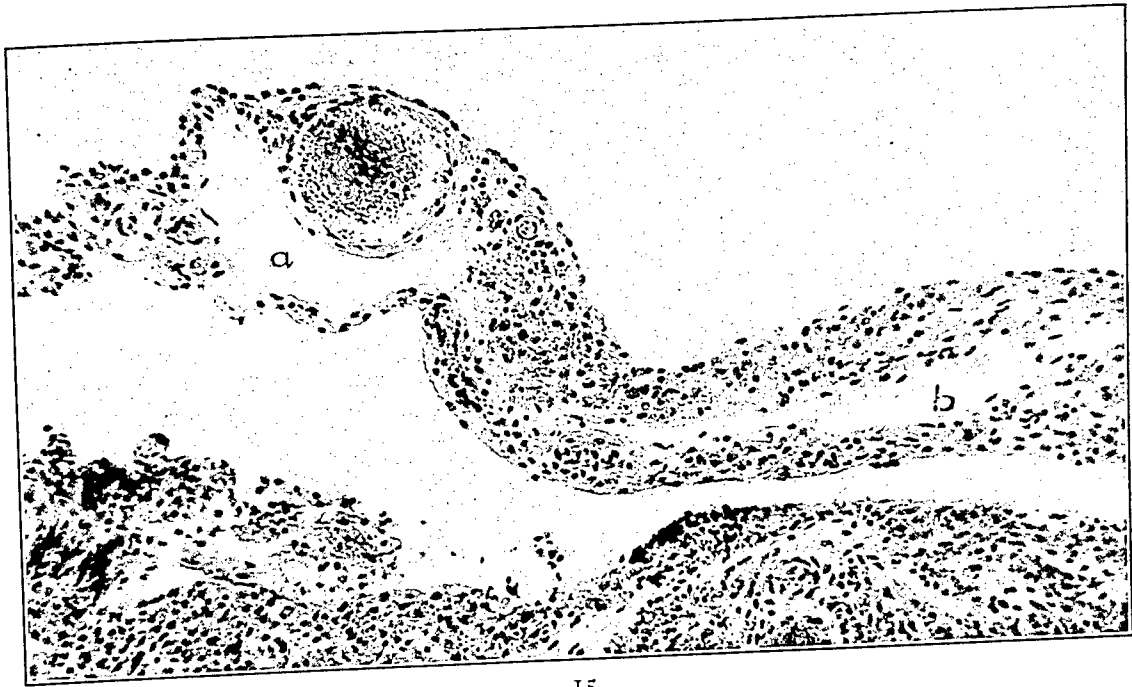
FIG. 15. Higher magnification of the slender strand of granulation tissue to the left of the two implants indicated by "b" in Fig. 13. Two portions "a" and "b" of a space running lengthwise of the strand are shown. Is it a lymph vessel or a space created by the incomplete fusion of two strands of granulation tissue? A dilated blood vessel appears above the letter "a."  $\times 130$ .



13



14



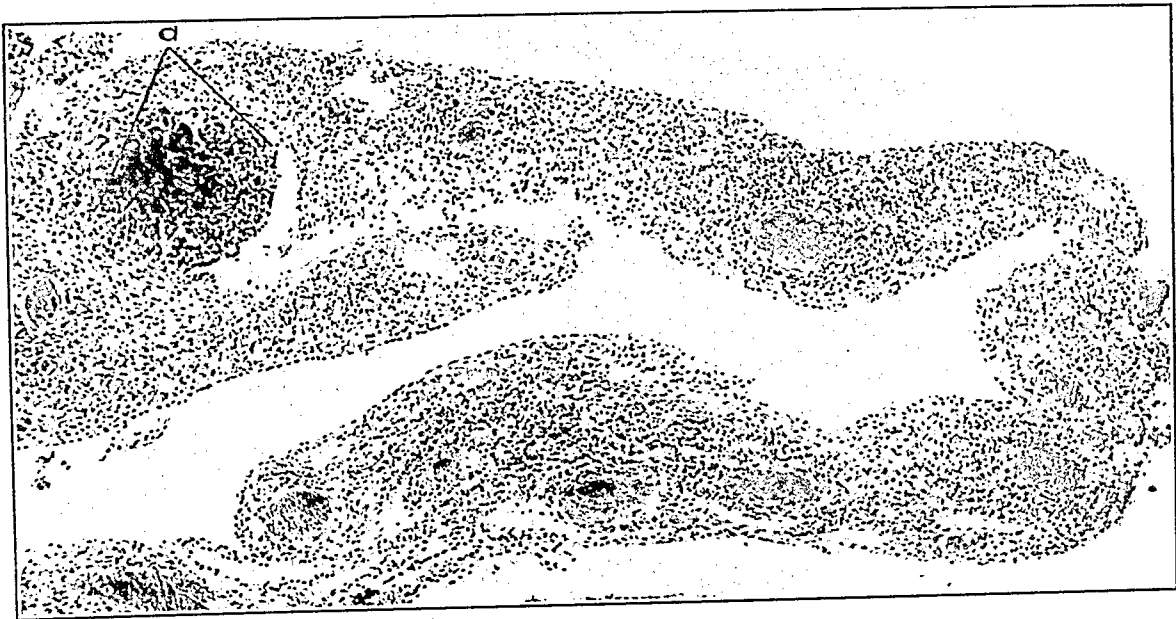
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PLATE 58

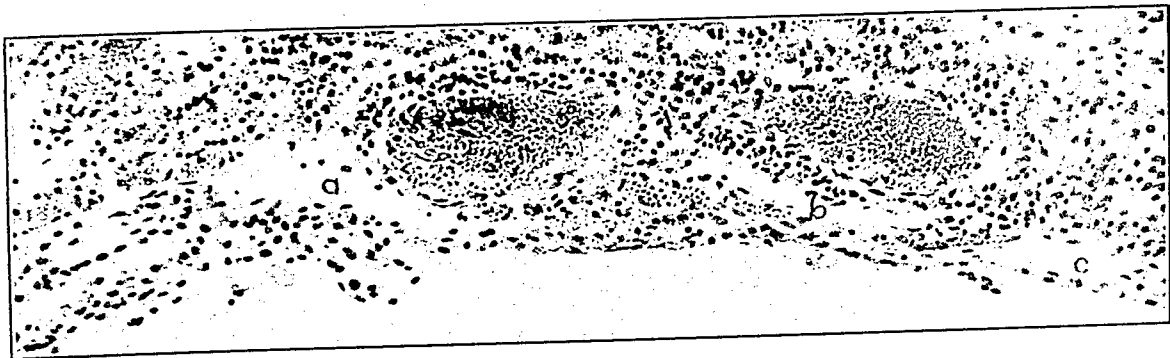
FIG. 16. The loop of granulation tissue in which implants "b" and "c" of Fig. 13 are situated, from another section. An implant "a" is situated in the upper limb of the loop. This is the one indicated by "c" of Fig. 13. It appears much larger in the present photomicrograph because the section passes through the center of the mass of cancer cells. The two implants indicated by "b" in Fig. 13 do not appear in this section. They are situated in the thickest portion of the lower limb of the loop. A large, tortuous, obliquely cut blood vessel appears in this loop. It is accompanied by a possible lymph vessel which is cut in a similar manner so that it simulates several vessels. For a higher magnification of these see the next illustration.  $\times 54$ .

FIG. 17. Higher magnification of the portion of the lower limb of the loop of granulation tissue, of Fig. 16, showing the large blood vessel, cut obliquely, and the accompanying possible lymph vessel. The lymph vessel is indicated by "a," "b" and "c." It is impossible to prove that this lymph vessel-like structure is not a space created by the rapidly growing granulation tissue. But if it is a lymph vessel it is newly formed and with newly formed blood vessels it helps to make up the capsule of the two implants indicated by "b" in Fig. 13.  $\times 130$ .

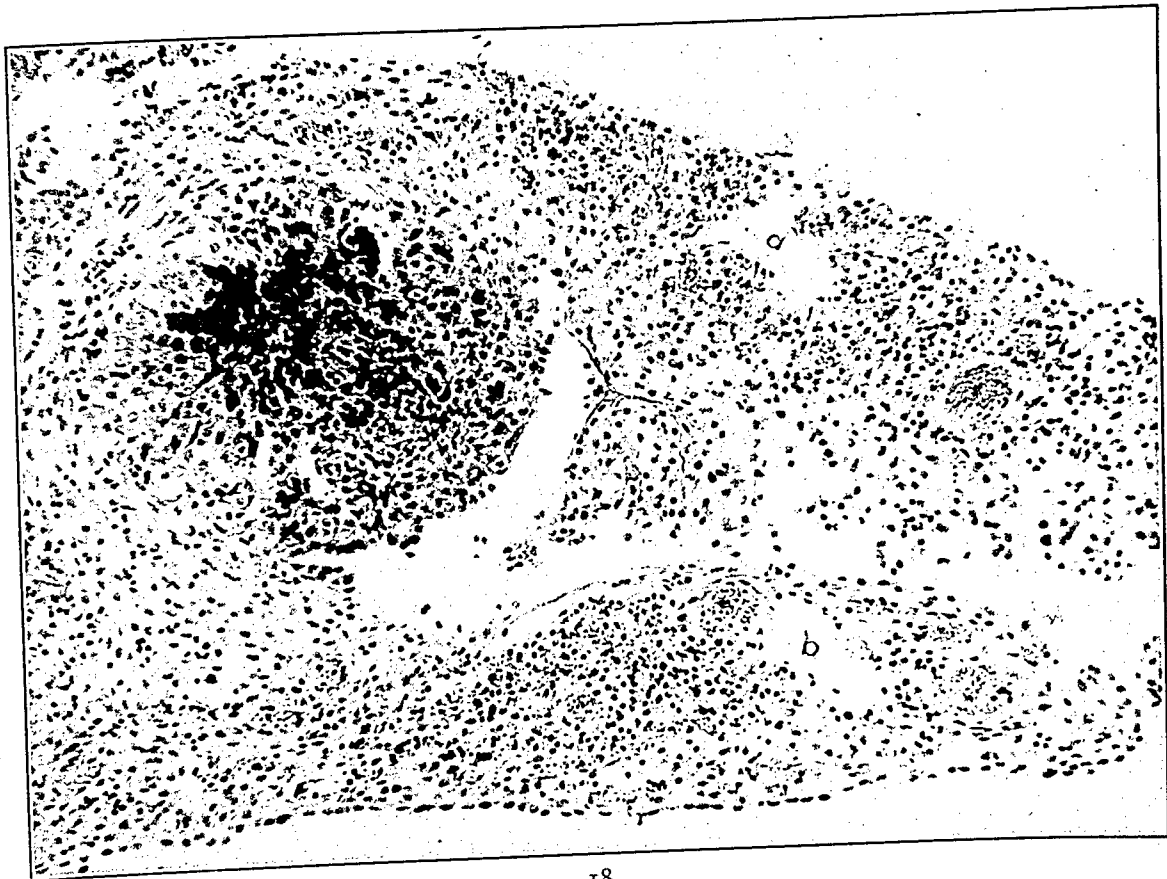
FIG. 18. Higher magnification of the portion of the upper limb of the loop of granulation tissue containing implant "a" of Fig. 16. Rapidly growing granulation tissue is encapsulating (swallowing) the clump of cancer cells implanted on its surface. Two spaces "a" and "b" are present in this tissue. Are they true lymph vessels or are they tissue spaces created by the incomplete filling in of rapidly growing granulation tissue?  $\times 130$ .



16



17

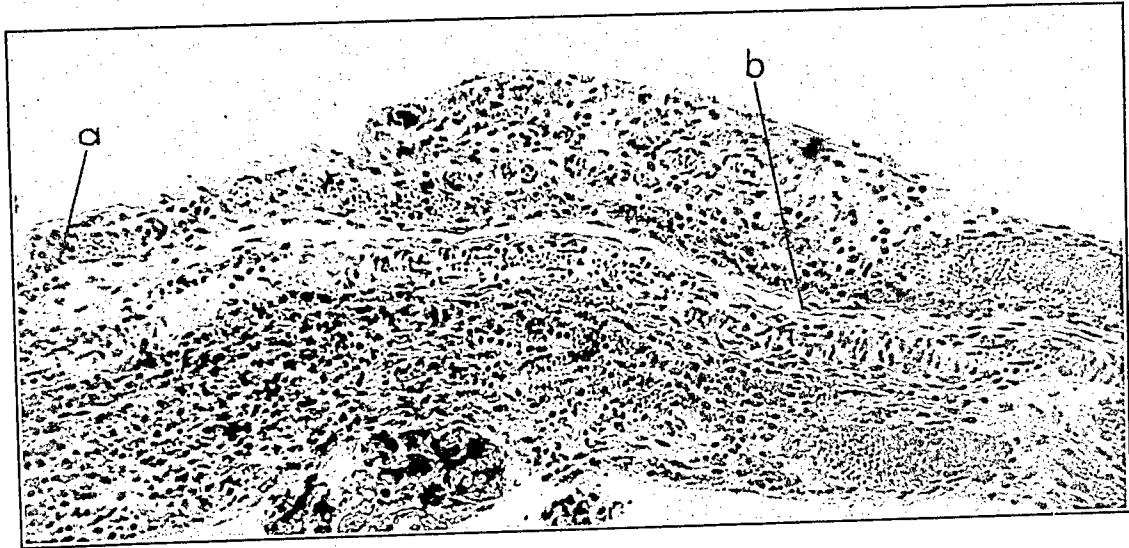


18

# PLATE 59

19. A portion of the strand of granulation tissue attached to the surface of the uterus indicated by "a" in Fig. 13 and just above the field shown in Fig. 14, from a nearby section. A space lined by endothelium-like cells and indicated by pointers "a" and "b" accompanies the blood vessels in their extension into this strand. Continuity with the possible lymph vessels ("a" and "b") shown in Fig. 14 cannot be established. However, they may be continuous but, because of the occlusion of the lumen from pressure, the vessel cannot be traced for its entire length.  $\times 130$ .
20. The same granulation tissue on the surface of the uterus shown in Fig. 13 from a section at some distance from the latter. At "c" carcinoma is implanted in newly formed tissue which constitutes the attachment of the strands of granulation tissue to the surface of the uterus. Carcinoma is encapsulated on the surface of the strand of granulation tissue at "a."  $\times 10$ .
21. Higher magnification of implant "c" of Fig. 20 and the tissue surrounding it. This tissue is a part of that indicated by "a" of Fig. 13 and is also shown in Fig. 14. The carcinoma is embedded in newly formed tissue which has grown out from the uterine wall through breaks in its mesothelial covering as indicated by "c" and "d." Newly formed blood vessels are present in this tissue and are accompanied by lymph vessel-like structures "a" and "b" similar to those shown in Fig. 14. Though the spaces "a" and "b" are continuous their continuity with preëxisting lymph vessels in the dense tissues of the uterine wall cannot be established. The presence of proved lymph vessels in another patch of granulation tissue from the same patient (see Figs. 3 to 12) is a strong indication that at least some of the lymph vessel-like structures just described are true lymph vessels even though their continuity with preëxisting vessels cannot be established because the latter are not evident. If the lymphatics had been injected direct continuity might have been demonstrated.  $\times 54$ .





19



20



21

Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

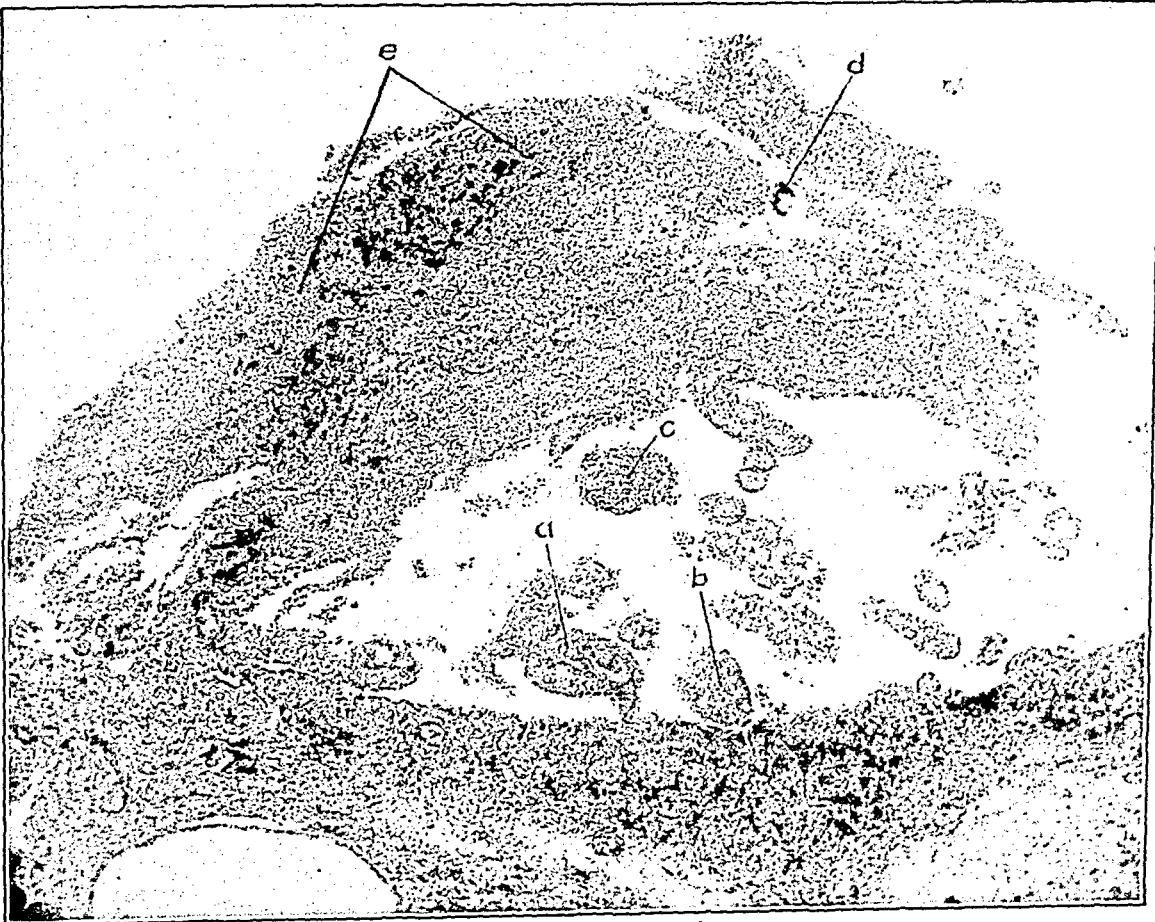
PLATE 60

FIG. 22. Granulation tissue on the surface of the Fallopian tube from a patient with carcinoma of both ovaries associated with a very early peritoneal carcinomatosis (Case 2). Carcinoma was not discovered in this patch of granulation tissue but might have been present as only a few sections were examined. The granulation tissue has grown out from the tubal wall through breaks in its mesothelial covering. In one place "m" the mesothelium is still intact. The granulation tissue has arched over this area leaving an opening between it and the surface of the tube. Spaces like these often become lined by mesothelium during the usual involution of the granulation tissue. When cancer cells become enmeshed in overlying granulation tissue, as well they may in this instance, a sessile polypoid implant with a fenestrated base may result (see Fig. 42). Judged newly formed lymph vessels "a" and "b" are present in one pillar of the arch of granulation tissue. For a higher magnification of this area see Fig. 24.  $\times 54$ .

FIG. 23. Granulation tissue which has arched over the surface of the Fallopian tube, from the same section shown in Fig. 22, but on the opposite side of the tube. The main pillar of the span is situated at the left. Additional supports or tethers appear, in the center and right, as tortuous slender strands of vascular granulation tissue (pedicles) extending from the surface of the tube to the span of granulation tissue above it. Small deeply stained masses, judged to be clumps of dead cancer cells, are enmeshed in the portion of the span of granulation tissue indicated by "e." A clump of living cancer cells is situated at "d." Lymph vessels cannot be detected in either the main pillar of the arch or the span. However, they are easily seen in the tethers (slender pedicles) "a," "b" and "c." For a higher magnification of pedicle "a," see Fig. 25. Later stages of implants similar to the one indicated in this illustration are shown in Figs. 85 and 89.  $\times 25$ .



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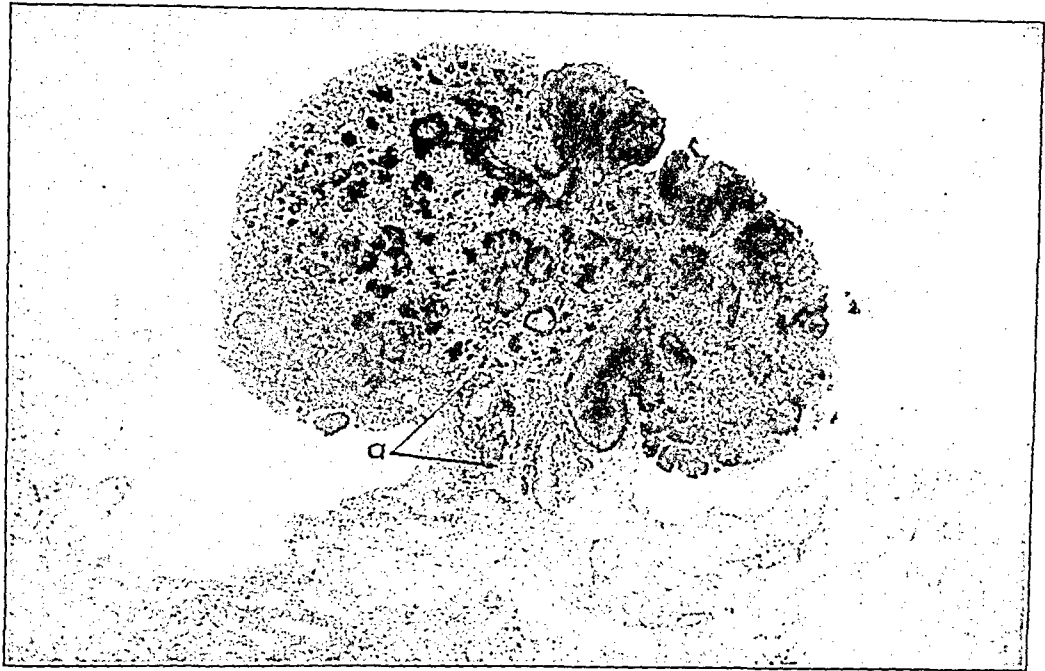
## PLATE 62

FIG. 26. A section of a mature predunculated polypoid implant on the vesico-uterine reflection of peritoneum, from a patient with carcinoma of both ovaries associated with peritoneal carcinomatosis (Case 3). Note that the kidney shaped tumor has abundant stroma. Blood vessels are quite evident, especially in its pedicle and hilum, and in other situations where the tissues of the stroma are less dense. Judged lymph vessels are present in the cortex of the implant opposite its hilum (see Fig. 27). These can be followed in the stroma to just above its midportion, Fig. 28, where they are lost apparently and then reappear in the stroma of the hilum and pedicle (see Figs. 29, 30 and 31). In this section lymph vessels can be detected only in the stroma of the hilum and pedicle. For a higher magnification of the area indicated by "a" see Fig. 30.  $\times 25$ .

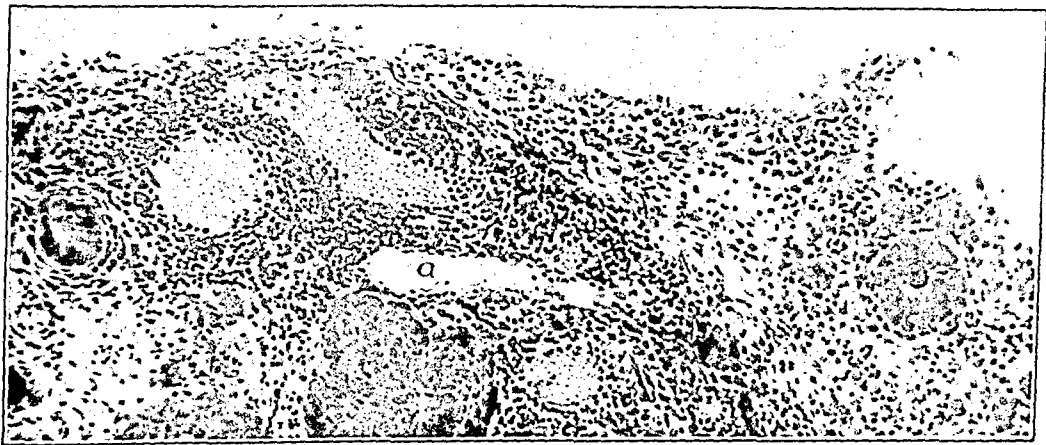
FIG. 27. A portion of the cortex opposite the hilum of the implant shown in Fig. 26, from another section. Masses of cancer cells are scattered throughout this portion of the tumor. Because of the relatively loose texture of the stroma the injected blood vessels are easily seen. Accompanying these is a vessel "a" lined by endothelium-like cells and without any blood in its lumen. I believe that it is a lymph vessel. Beneath this vessel is a mass of cancer cells.  $\times 130$ .

FIG. 28. A small area just above the center of the implant shown in Fig. 26, from another section. Blood vessels are not evident. However, an oblique section of a very thin walled vessel lined by endothelium-like cells appears in the center of the photomicrograph. I believe that it is a lymph vessel and possibly is continuous with the one shown in Fig. 27.  $\times 130$ .

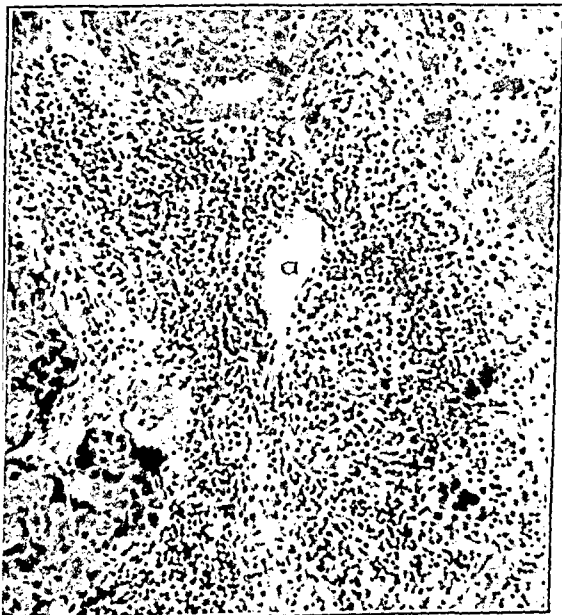
FIG. 29. A portion of the stroma of the hilum of the implant shown in Fig. 26, from another section. A thin walled vessel "a," similar to the preceding ones, is shown in cross-section with a few lymphocytes in its lumen. Blood vessels are about it. It is an easy matter to distinguish a lymph vessel from veins "b" and "c" when the latter contain blood.  $\times 130$ .



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PLATE 63

FIG. 30. Higher magnification of a portion of the stroma of the hilum and pedicle of the implant indicated by "a" in Fig. 26. An oval mass of lymphocytes is present in this area with a vein "v" in its upper portion and two judged lymph vessels "a" and "b" beneath it.  $\times 130$ .

FIG. 31. A portion of the base of the pedicle of the implant shown in Fig. 26, from another section. A dilated vein "v" appears in cross-section with two judged lymph vessels "a" and "b," or portions of one vessel, below and to the right of it. Both the blood and lymph vessels may have been in this situation before the implant developed. It is a relatively easy matter to follow newly formed blood vessels filled with blood, from preëxisting blood vessels of the peritoneum through the pedicle of the tumor and into its stroma. On the other hand, it is impossible to follow the non-injected lymph vessels. The fact that judged lymph vessels are found about the blood vessels in Figs. 27, 29 and 30 is a very strong indication that they accompany the newly formed blood vessels in the development of the granulation tissue which later forms the stroma of the mature implant. Therefore, I believe that this mature implant (tumor) contains newly formed lymph vessels as well as newly formed blood vessels in its stroma.  $\times 130$ .

FIG. 32. The base of the implant, its pedicle and the peritoneum beneath it, from the same section pictured in Fig. 31 (lower magnification), showing the relation of the blood vessel "v" to the implant and the peritoneum beneath it.  $\times 54$ .



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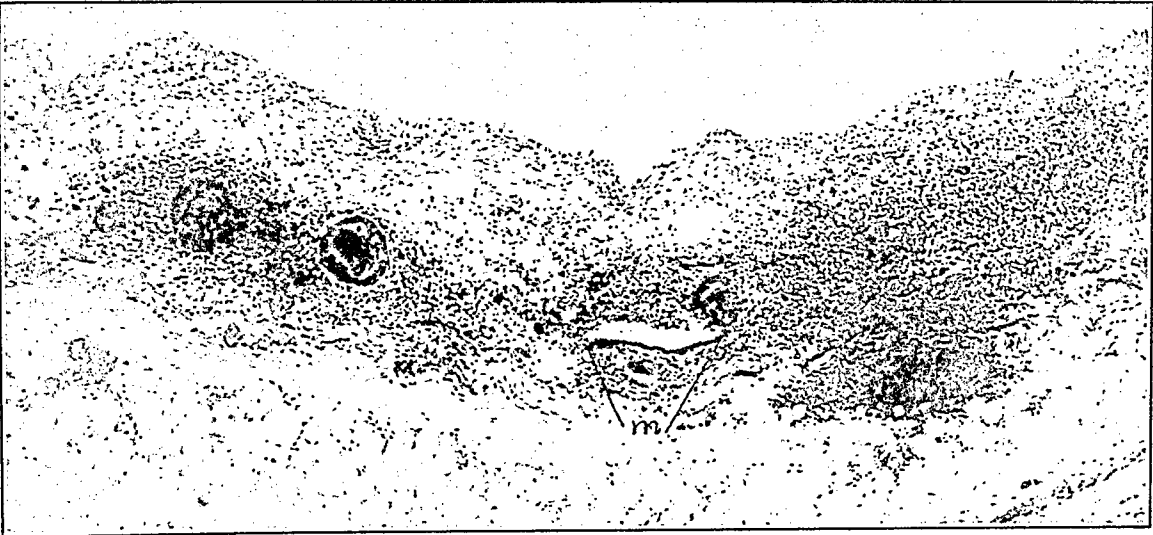
# PLATE 64

FIG. 33. A section of the thickened parietal peritoneum with carcinoma embedded in it, from the same patient (Case 3). The "key" to the pathogenesis of this lesion lies in the small strip of mesothelium "m" near the center of the photomicrograph. This represents the level of the surface of the peritoneum prior to the implantation of the cancer cells. The latter became enmeshed in granulation tissue which developed on the surface of the peritoneum through breaks in its mesothelial covering. This granulation tissue has arched over the portion of the surface of the peritoneum covered by mesothelium; therefore, all of the tissue above the level of this mesothelium is newly formed.  $\times 54$ .

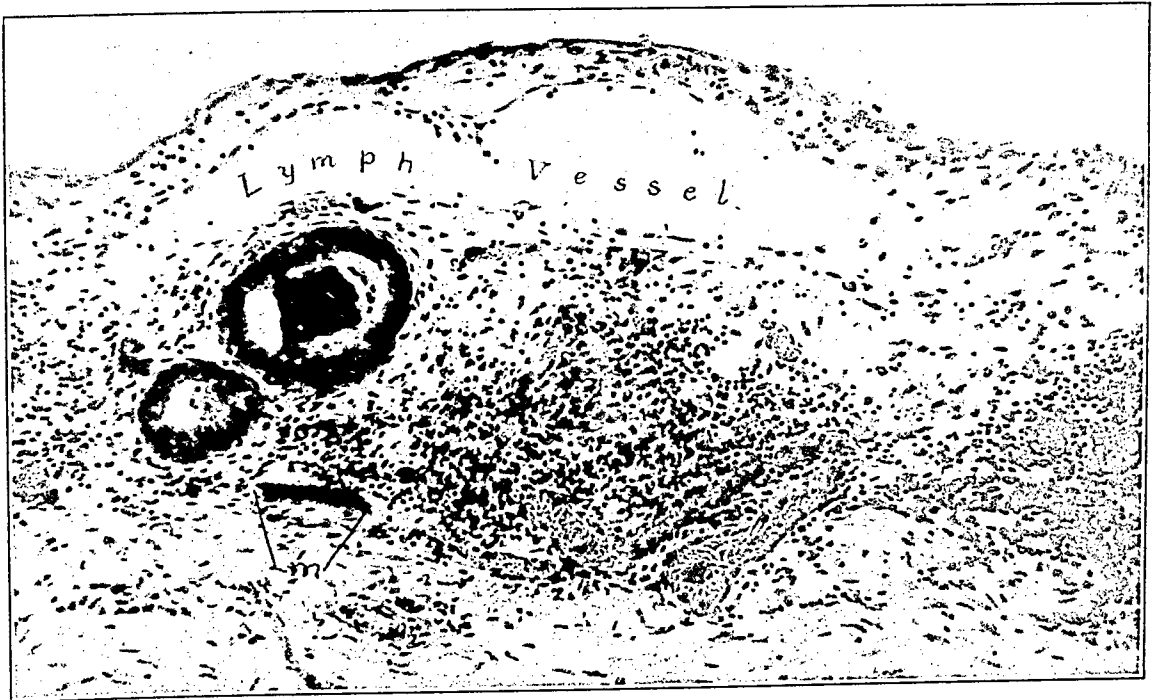
FIG. 34. Another section from the same block of tissue shown in Fig. 33. The conditions in this field, in many ways, are similar to those shown in the preceding photomicrograph. Carcinoma is embedded in the peritoneum which has become thickened by the development of granulation tissue on its surface through breaks in its mesothelial covering. The intact mesothelium "m," as in the section shown in Fig. 33, represents the original level of the surface of the peritoneum. All of the tissue above this level is newly formed. In it is situated a dilated channel which presents the histological structure of a lymph vessel. A few lymphocytes are present in its lumen. Note that the tissue about this vessel is loose, possibly edematous, which might permit or even cause the dilatation of lymph vessels in it. This vessel may be followed through several sections in the series but its continuity with preëxisting lymphatics cannot be established. However I believe that it may well be a newly formed lymph vessel. Compare with the newly formed lymph vessel shown in Fig. 7 and the preëxisting lymphatic shown in Fig. 36.  $\times 130$ .

FIG. 35. A section of the thickened vesico-uterine reflection of peritoneum from the same block of tissue in which the pedunculated implant described in Fig. 26 is situated. The conditions in this section are quite similar to those shown in the two preceding photomicrographs except for the fact that a patch of intact mesothelium is lacking by which one may determine the original surface level of the peritoneum. However I believe that all of the tissue in which the carcinoma is situated is newly formed (compare this section with Fig. 33). Judged newly formed lymph vessels "a" and "b," similar to those shown in Figs. 27 and 28, are situated in this tissue. To the right an early implantation of clumps of cancer cells in newly formed granulation tissue is being added to the surface of the thickened peritoneum.  $\times 54$ .

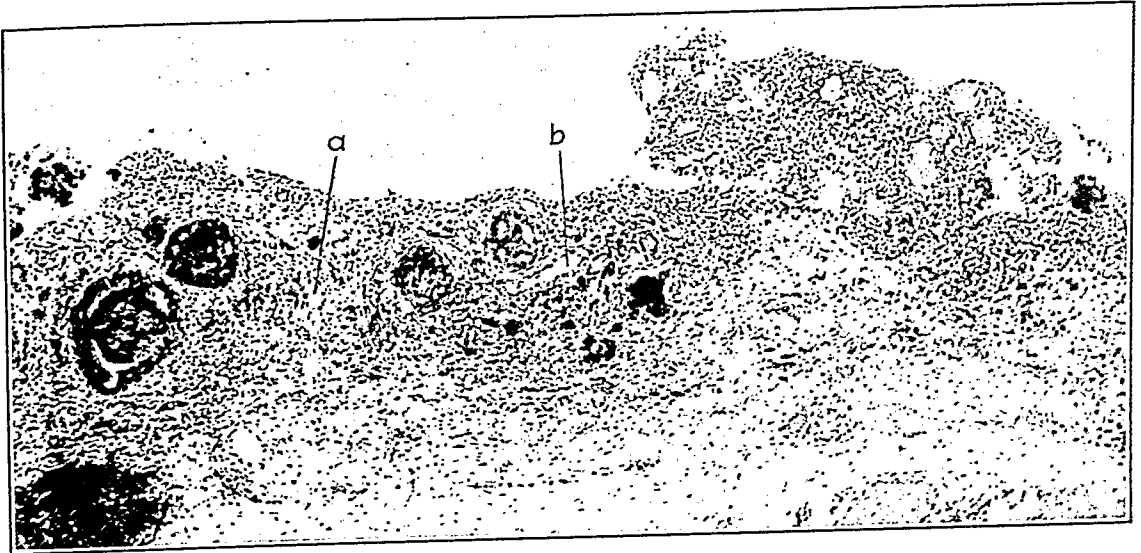




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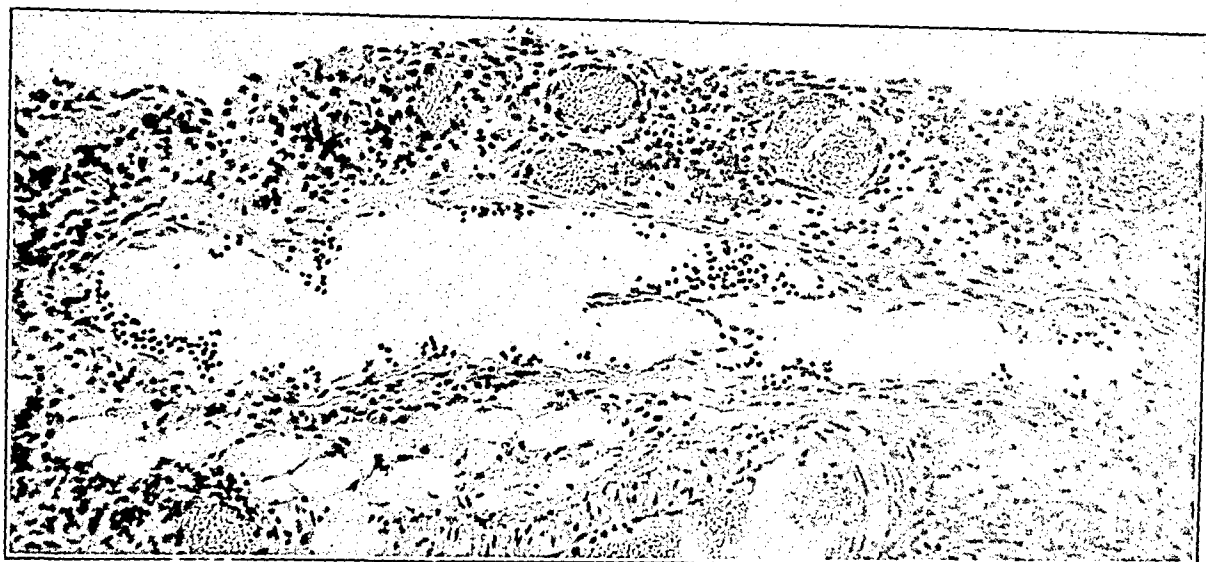
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PLATE 65

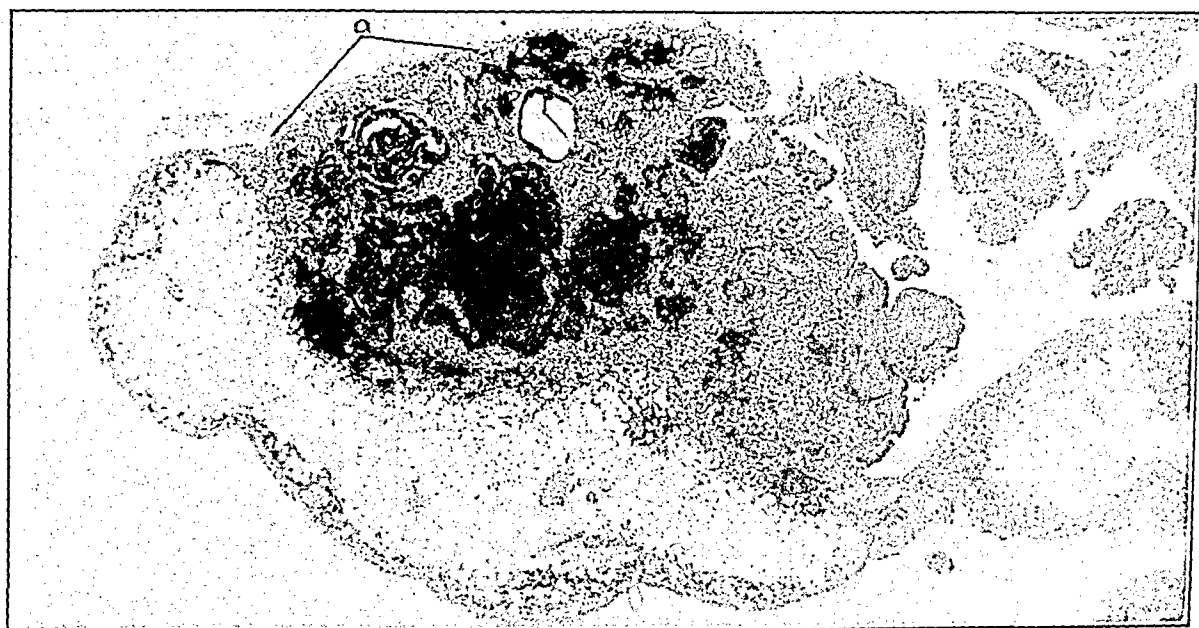
FIG. 36. A section of a portion of the omentum near its free margin, from the same patient (Case 3). Many carcinomatous metastases are present in other situations in this omentum. A large lymph vessel, with valves and lymphocytes in its lumen, is shown. This vessel is probably not newly formed. Compare with the newly formed lymphatic shown in Fig. 34 and also in Figs. 7 and 10.  $\times 130$ .

FIG. 37. A section of the same omentum shown in Fig. 36 with a large sessile polypoid implant on its surface. All of the tissue in which carcinoma is situated is newly formed. Because the stroma of the base of the implant is dense, newly formed lymph vessels cannot be detected in this portion of the tumor. However they can be seen in the less dense and more vascular cortex (see the next illustration).  $\times 25$ .

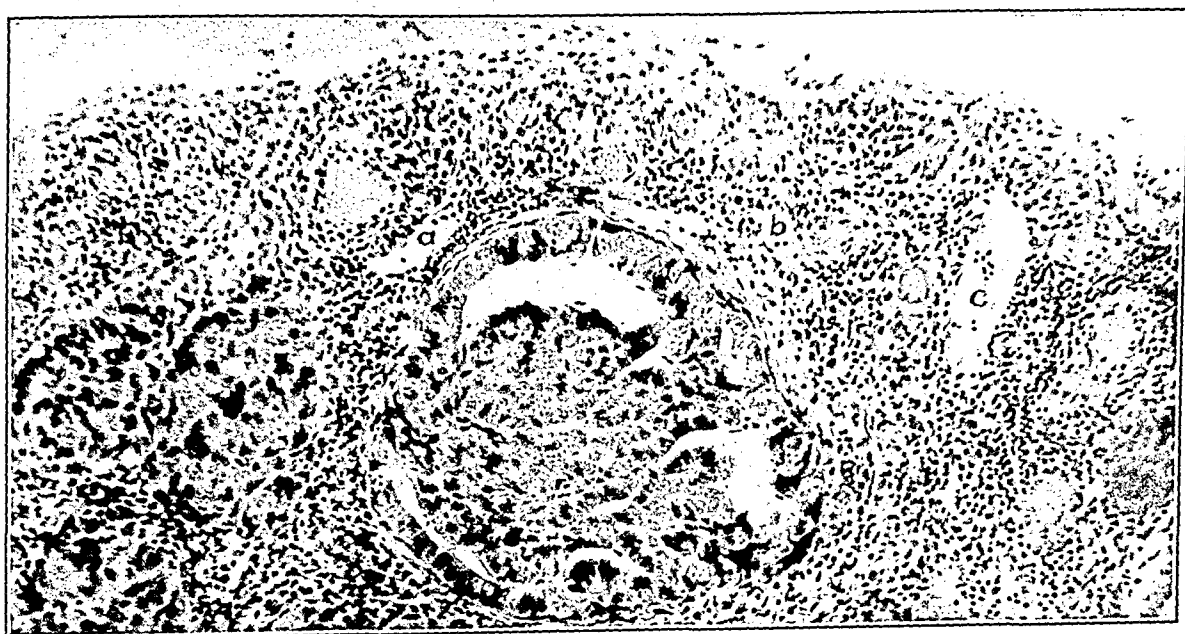
FIG. 38. Higher magnification of the portion of the implant, indicated by "a," in the preceding photomicrograph. The stroma of the tumor in this situation is relatively loose and contains newly formed blood vessels and likewise judged newly formed lymph vessels "a," "b" and "c." Lymphocytes are present in the lumina of these lymph vessels. The structure of these vessels is identical with that of those shown in Figs. 27, 28, 29, 30 and 35.  $\times 130$ .



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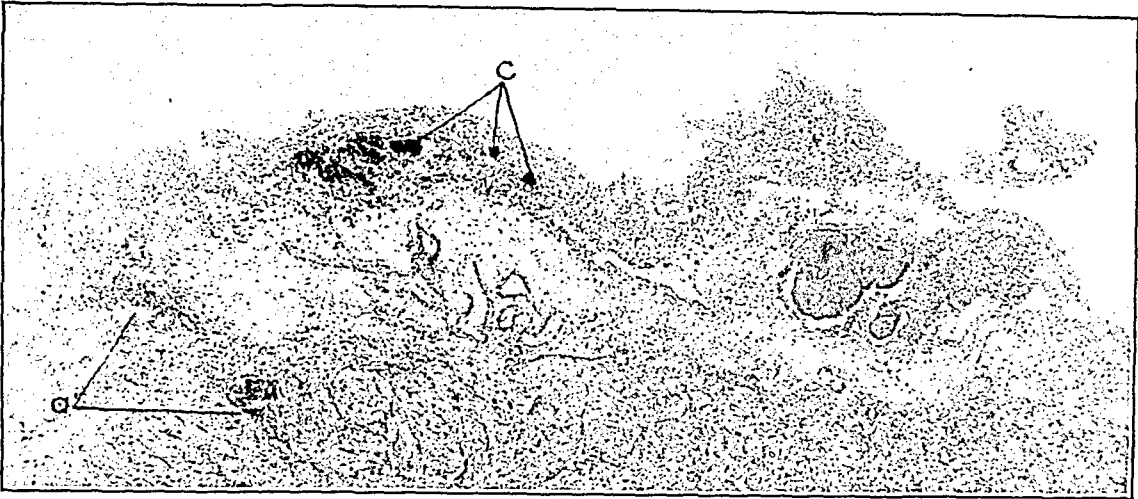
PLATE 66

FIG. 39. A small patch of early granulation tissue on the lower portion of the anterior wall of the uterus from a patient with carcinoma of both ovaries associated with peritoneal carcinomatosis (Case 4). Carcinoma is embedded in this tissue at the right. Clumps of additional cancer cells "c" are becoming implanted on the surface of this granulation tissue. A judged newly formed lymph vessel arising from a preëxisting lymph vessel is indicated by "a."  $\times 10$ .

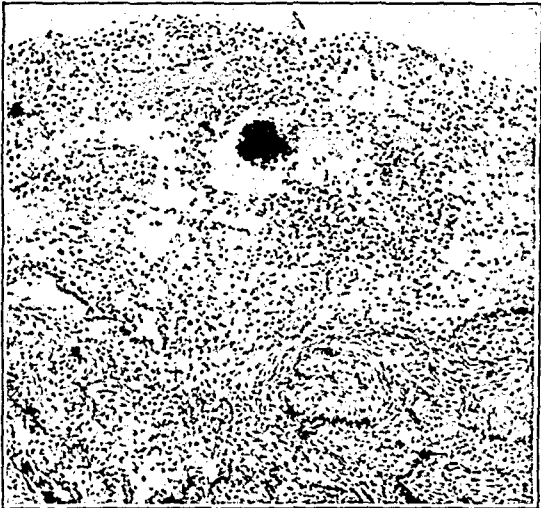
FIG. 40. The central portion of the granulation tissue shown in Fig. 39, but from another section. The granulation tissue has grown out through a break in the mesothelial covering of the uterus. A clump of cancer cells is becoming enmeshed in this tissue.  $\times 54$ .

FIG. 41. Higher magnification of the area of granulation tissue, containing carcinoma, shown in Fig. 39, but from another section. This represents the most mature implantation of carcinoma found in this patch of granulation tissue and may well have been the initial exciting cause of the granulation tissue about it. Granulation tissue is pouring out through a break in the mesothelial covering of the uterus. A possible newly formed lymph vessel accompanying newly formed blood vessels is indicated by "a." This cannot be followed farther into the granulation tissue nor can its continuity with underlying preëxisting lymph vessels be established. The latter cannot be detected in this situation.  $\times 54$ .

FIG. 42. A sessile polypoid implant situated on the surface of the vesico-uterine reflection of peritoneum below the patch of granulation tissue shown in Fig. 39. It may well represent a later stage of the condition shown in Fig. 41. Very few blood vessels and no lymph vessels can be detected in the dense mass of carcinoma in this section. On the other hand these structures can be detected in the loose granulation tissue at either end.  $\times 20$ .



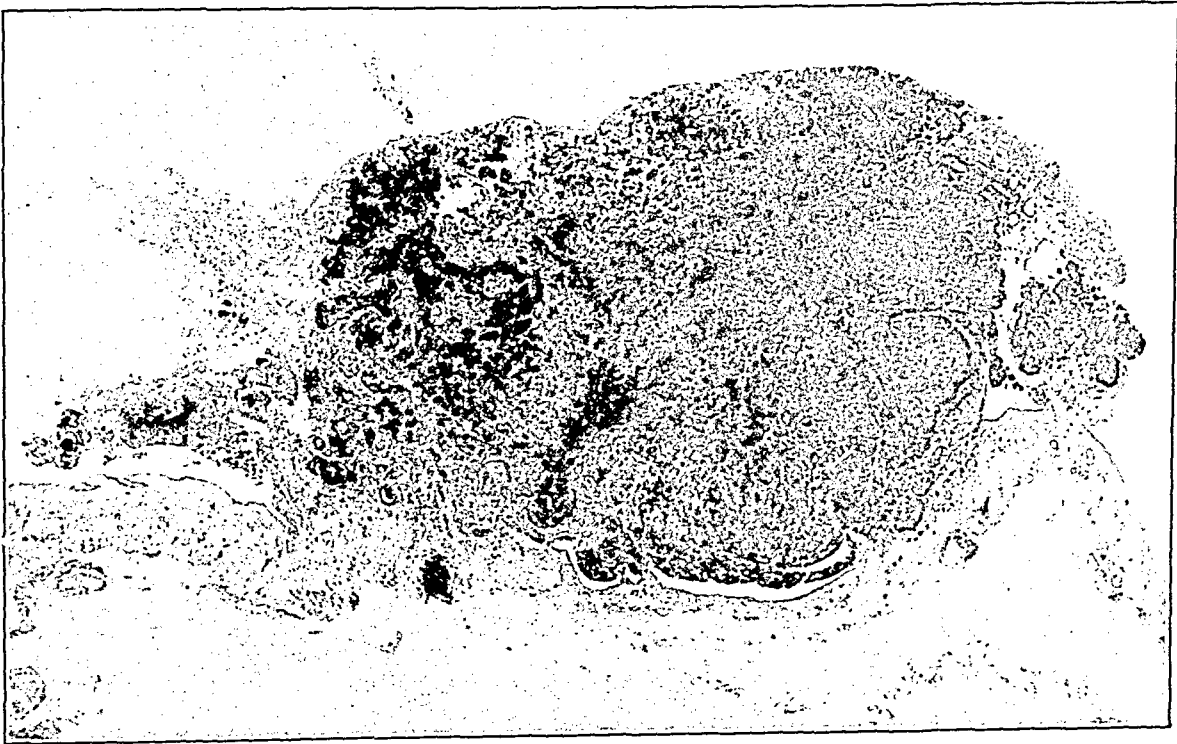
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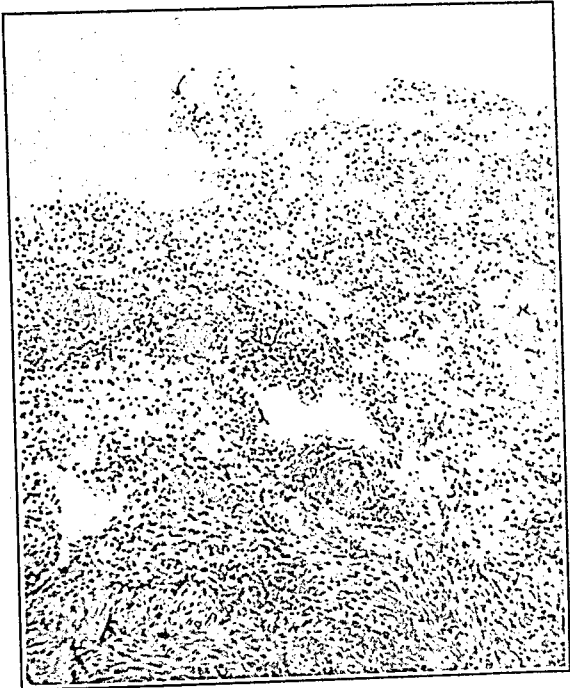
42

PLATE 67

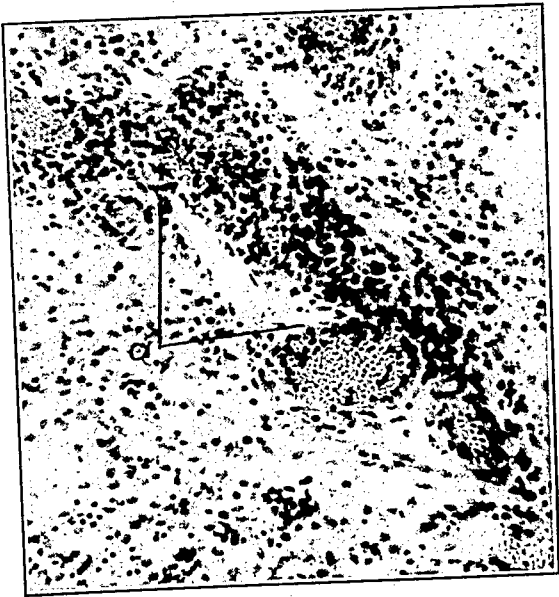
- FIG. 43. Higher magnification of the judged newly formed lymph vessel indicated by "a" of Fig. 39. A clump of lymphocytes, with lymph vessels near them, is present in preëxisting tissue beneath the granulation tissue (see Fig. 47). At the upper arm of the pointer "a" is a judged newly formed lymph vessel accompanying newly formed blood vessels (see Fig. 45). I believe that this lymph vessel arises from the preëxisting lymph vessel indicated by the lower arm of pointer "a."  $\times 54$ .
- FIG. 44. A blood vessel accompanied by newly formed lymph vessels nearly midway between the lymph vessels indicated between the areas of pointer "a" in Fig. 43. I believe that it represents a connecting link between the two groups of vessels (see Fig. 46).  $\times 54$ .
- FIG. 45. Higher magnification of the judged newly formed lymph vessel "a," accompanying newly formed blood vessels, indicated by the upper arm of the pointer in Fig. 43.  $\times 130$ .
- FIG. 46. Higher magnification of the vessels shown in Fig. 44. The judged newly formed lymph vessels are clustered about a newly formed blood vessel (compare with Fig. 45). This granulation tissue with its newly formed blood and lymph vessels is providing the stroma for the growing implant (see Fig. 39). I believe that the vessels in Fig. 45 are but continuations of those shown in this photomicrograph. It seems logical to conjecture that carcinoma about these lymph vessels might easily invade them and thus more readily gain access to the lymphatic circulation of the host.  $\times 130$ .
- FIG. 47. Higher magnification of the preëxisting lymphatics indicated by the lower area of pointer "a" in Fig. 43.  $\times 130$ .
- FIG. 48. The same lymphatics shown in Fig. 47, from another section in the series. A mass of lymphocytes covered by endothelium is bulging into the dilated lymph vessel. Sections farther on in the series show the same vessel without the lymphocytes.  $\times 130$ .



43



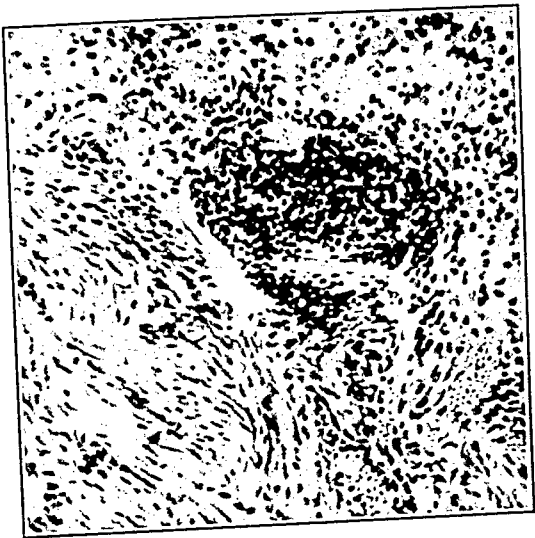
44



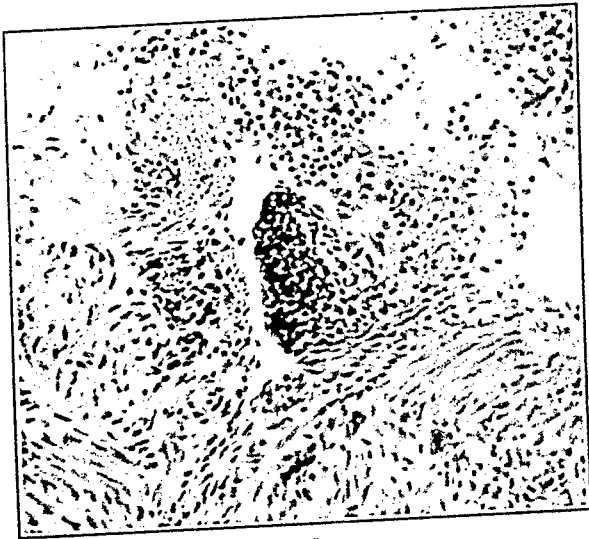
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## PLATE 68

FIG. 49. A section of sediment obtained by centrifugalizing the ascitic fluid present in the patient with the peritoneal implants just described. Clumps of cancer cells can be easily distinguished from the exfoliated mesothelial cells about them.  $\times 130$ .

FIG. 50. The tip of a polypoid outgrowth of granulation tissue which is attached to the peritoneum at the base of the pedicle of the large pedunculated implant shown in Fig. 52, but from another section. Clumps of cancer cells, similar to those shown in Fig. 49, are becoming enmeshed in the tip of this tissue, its youngest and most actively growing portion. They are not found in any other portion of this outgrowth of granulation tissue.  $\times 54$ .

FIG. 51. Another outgrowth of granulation tissue similar in its situation to the preceding one and possibly of the same age. It presents a later stage of the process of cancer cell implantation just shown. Granulation tissue is growing over and encapsulating the cancer cells which have also grown. I believe the phenomenon shown in these two photomicrographs represents the implantation of cancer cells in preëxisting granulation tissue which developed as the result of the stimulation caused by cancer cells escaping into the peritoneal cavity. The granulation tissue in both outgrowths is abundantly supplied with newly formed blood vessels. Yet in spite of a careful study of many sections of these outgrowths, lymph vessels cannot be recognized in them. Either they are not present or, if present, their walls are collapsed, the nuclei of their endothelial linings being indistinguishable from those of cells surrounding them.  $\times 54$ .





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PLATE 69

FIG. 52. A section of the vesico-uterine reflection of peritoneum with three polypoid implants on its surface (Case 4). A large sessile one (also shown in Fig. 42) appears at the right, a large pedunculated one at the left and a small one to the right of this. The pedicle of the small implant has an origin common with that of the larger one (see Fig. 53).  $\times 10$ .

FIG. 53. The union of the pedicles of the two polypoid implants shown in Fig. 52, but from another section. The pedicle of the larger one contains two masses of lymphocytes indicated by the pointer "l. t." The pedicle of the smaller implant, approaching the latter from the right, contains a channel "l. c." filled with lymphocytes. This channel is either a tissue space or a lymph vessel (see Fig. 55).  $\times 54$ .

FIG. 54. Higher magnification of the mass of lymphocytes indicated by the upper arm of the pointer "l. t." in Fig. 53. These lymphocytes are nearly surrounded by blood vessels. A clump of epithelium-like cells is situated in the lower portion of this mass of lymphocytes. These cells resemble very closely the cells in portions of the carcinoma in the implant above it (see Figs. 56 and 57). Also compare with the carcinoma shown in Fig. 62. If these are cancer cells they must have reached their present situation by way of lymphatics from carcinoma in the larger implant, Figs. 56 and 57, or they were deposited here during the development of the implant.  $\times 130$ .

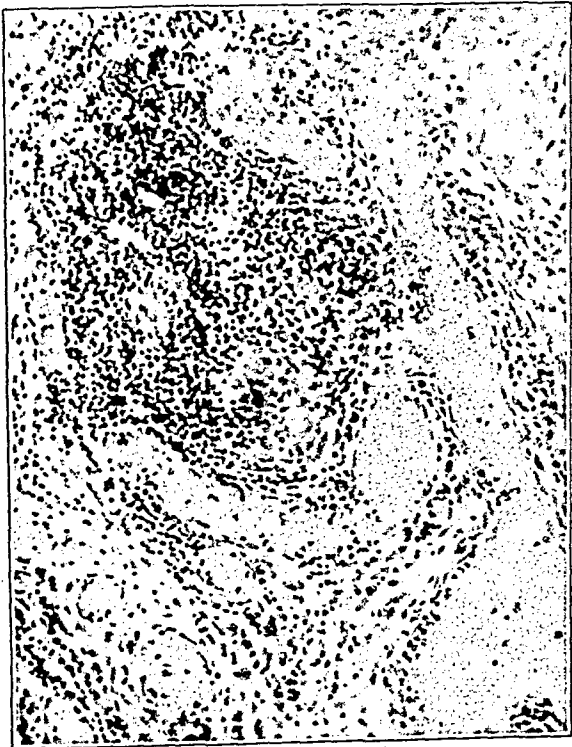
FIG. 55. Higher magnification of the tissue space or lymph vessel containing lymphocytes indicated by "l. c." of Fig. 53. Lymph vessels cannot be detected in many sections of the pedicles of either implant. However they may be present but because of the compression of their lumina they cannot be distinguished from the tissue surrounding them.  $\times 130$ .



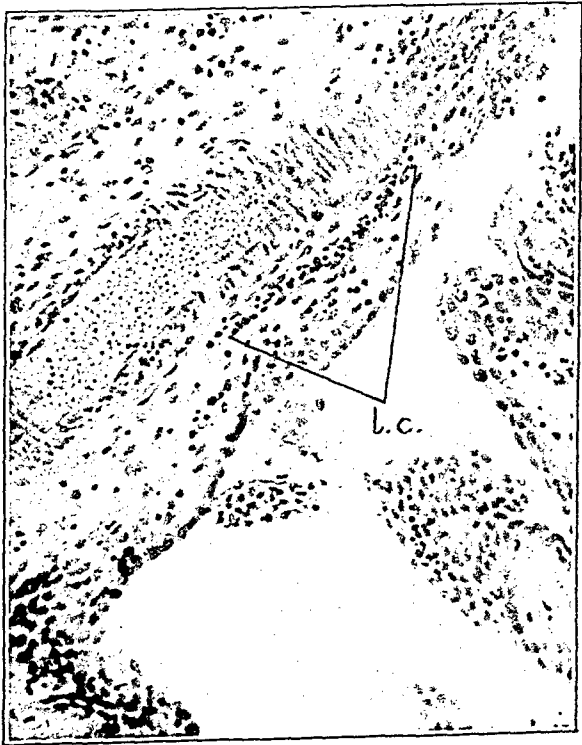
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53



54



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PLATE 70

FIG. 56. The lower portion of the larger implant and the upper part of its pedicle. Two possible lymph vessels are indicated by "a" and "b." Lymph vessel "a" can be seen in other sections but can be followed for only a short distance in either direction.  $\times 54$ .

FIG. 57. Higher magnification of the possible lymph vessel accompanying a blood vessel to its left and indicated by "b" in Fig. 56. It is impossible to follow this vessel or tissue space in either direction or to ascertain that the carcinoma at the right has invaded it. The carcinoma may well have compressed or even destroyed the vessel at other levels.  $\times 130$ .

FIG. 58. An early pedunculated polypoid implant which is situated to the left of the sessile polypoid implant shown in Fig. 52, from another section. Carcinoma may be found embedded in other portions of this tissue. Two possible lymph vessels in granulation tissue about the base of the implant are indicated by "a" and "b." Both of these vessels can be followed in many sections. Vessel "b" extends well up into the tip of the implant and also down to its base. The continuity between these vessels and pre-existing vessels in the peritoneum beneath the granulation tissue cannot be established. Peritoneal lymph vessels cannot be detected in this situation.  $\times 25$ .

FIG. 59. Higher magnification of the possible lymph vessel indicated by "b" in Fig. 58. The oval nuclei which appear to be in the lumen of the vessel may be the nuclei of endothelial cells lining the curved portion of its wall. Compare the nuclei of the cells lining this channel with those pictured in the lymph vessels of Fig. 2. I believe that it may be a lymph vessel. The only other possibility is that this is a space arising from the incomplete fusion of a slender strand of granulation tissue with a larger strand to the left (compare with Figs. 61 and 62).  $\times 130$ .



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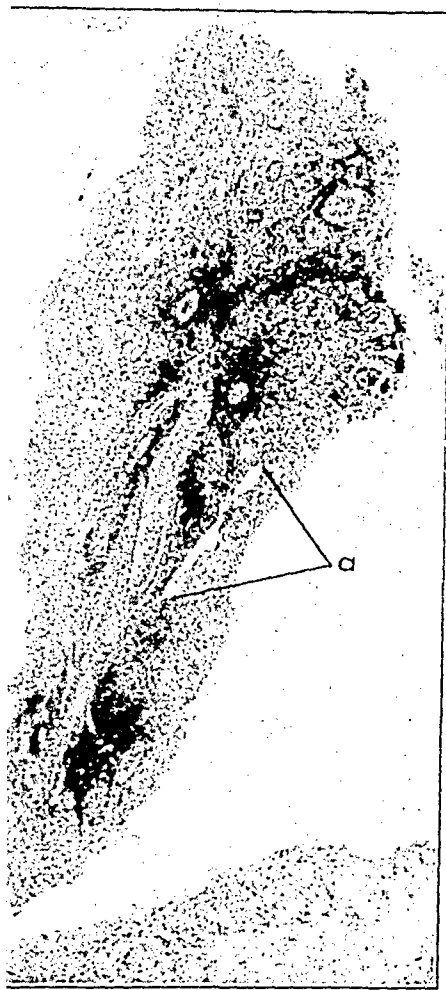
59

Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

PLATE 71

- FIG. 60. A longitudinal section of the smaller pedunculated polypoid implant shown in Fig. 52, but from another section. Carcinoma is situated in the upper portion of this tissue. A possible lymph vessel is indicated by "a." This can be followed, in other sections, for some distance into the upper portion of the implant but it cannot be traced into the pedicle.  $\times 25$ .
- FIG. 61. Higher magnification of the possible lymph vessel indicated by "a" in Fig. 60. A few lymphocytes are present in its lumen. It is almost an exact duplicate of the possible lymph vessel shown in Fig. 59 and must have had a similar origin (compare with Figs. 2, 3, 7 and 34).  $\times 130$ .
- FIG. 62. Farther extension, "a," into the upper portion of the implant of the possible lymph vessel shown in Fig. 61. This is as far as it can be traced in this direction. Here it is surrounded by lymphocytes and carcinoma very similar to the cancer-like cells shown in Fig. 54. Some of the carcinoma is very close to this vessel. It is conceivable that the vessel cannot be followed farther because its lumen is compressed by the carcinoma. It is possible that a space created by the incomplete fusion of two strands of granulation tissue, as suggested in Fig. 59, may simulate the structure indicated by "a." However, I believe that this structure may well be a lymph vessel.  $\times 130$ .



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62

Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

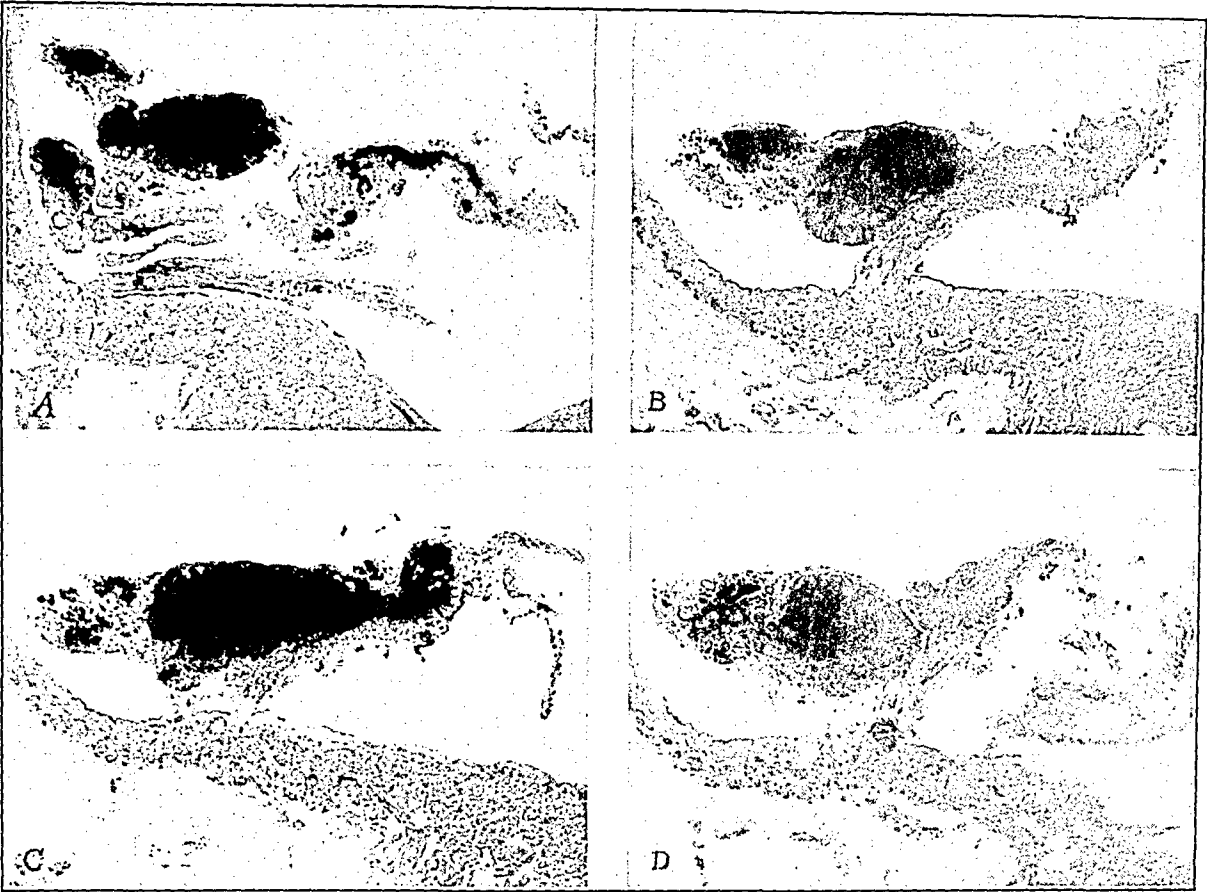
# PLATE 72

FIG. 63. Four photomicrographs "A," "B," "C" and "D," from a series of sections of a mature pedunculated polypoid implant with many pedicles, situated on the vesico-uterine reflection of peritoneum near its attachment to the uterus (Case 4). "A" shows carcinoma implanted in strands of granulation tissue arising from the peritoneum beneath them. The condition here may well represent a later stage of that shown in Figs. 13 and 20. In "B," from a section some distance from "A" in the series, one of the several pedicles of this implant, which now resembles a crouching bird, appears. In "C" with two additional pedicles the bird-like implant of "B" now resembles a prehistoric mammal. In "D" the prehistoric mammal of "C" is changed into a squirrel with a long, curved, bushy tail and its forefeet lifted as though it were about to jump. Possible newly formed lymph vessels as well as newly formed blood vessels may be found in all of these pedicles.  $\times 8$ .

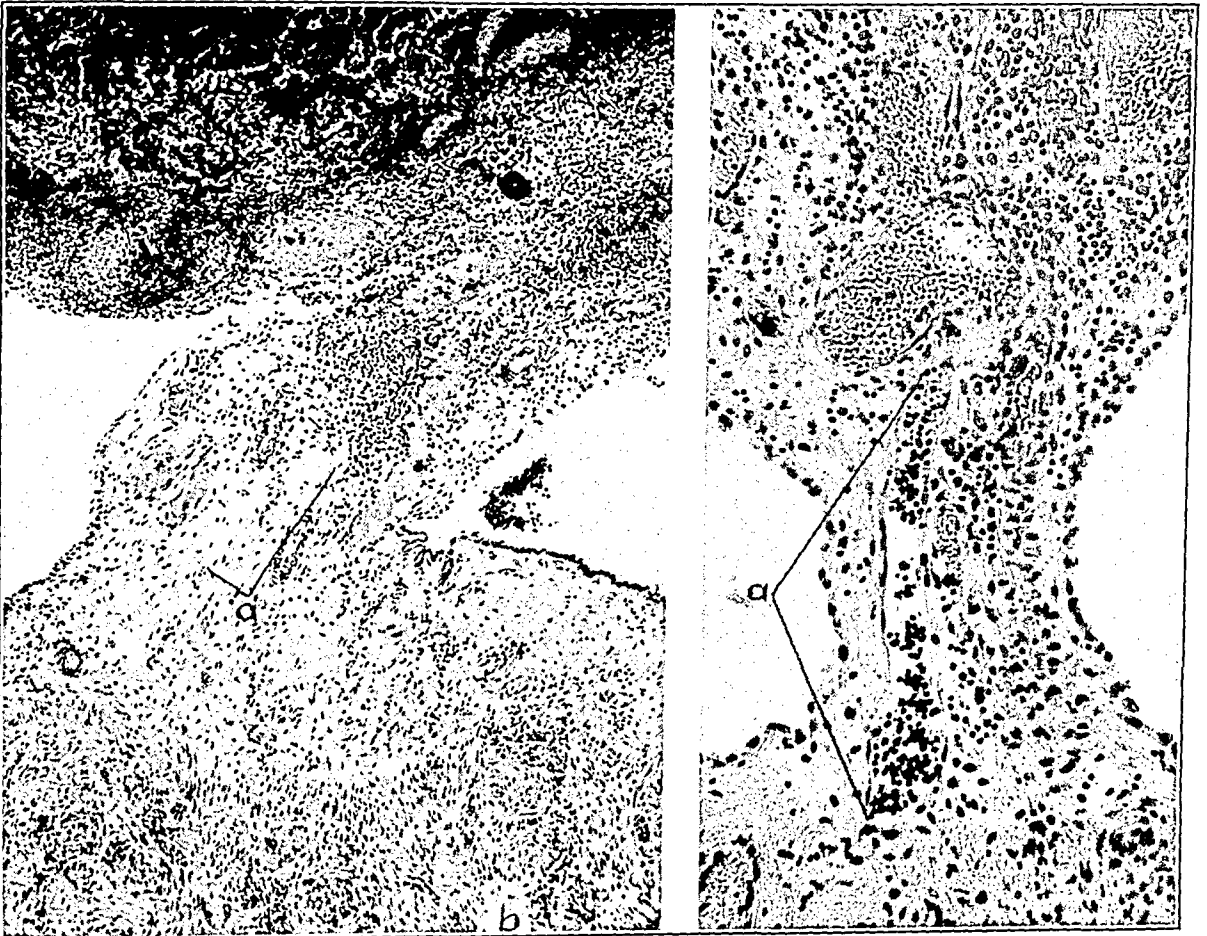
FIG. 64. Higher magnification of the pedicle of the implant shown in "B" of Fig. 63. A lymph vessel in the subperitoneal tissues is indicated by "b." Judged lymph vessels "a" are situated in the base of the pedicle. These apparently accompany blood vessels. The latter can be followed through the pedicle into the base of the implant. In this section the lymph vessels can be followed for only a short distance into the pedicle.  $\times 54$ .

FIG. 65. Higher magnification of the left of the two pedicles of the implant shown in "C" of Fig. 63, from another section. A possible lymph vessel is indicated by "a." The greater portion of its lumen is filled with lymphocytes. A few red blood corpuscles occupy its upper portion. It is lined by endothelium-like cells and accompanies the newly formed blood vessels in their extension through the pedicle of the tumor. The lower end of this vessel may well be preëxisting but that portion in the pedicle must be newly formed. The vessel cannot be traced farther into the implant.  $\times 130$ .





63



64

65

Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

PLATE 73

FIG. 66. A broad pedicle of the implant shown in Fig. 63 and presenting the fused portion of the two pedicles shown in "c" of that illustration. A lymph vessel in the subperitoneal tissues is indicated by "f." Small, judged newly formed lymph vessels "b," "c," "d" and "e" are present in this pedicle. A similar vessel "a" is situated just below the advancing carcinoma. The continuity of the judged lymph vessels in this pedicle with preëxisting lymph vessels in the peritoneum is suggested in other sections but cannot be definitely established.  $\times 54$ .

FIG. 67. Higher magnification of the right of the two pedicles of the implant shown in "c" of Fig. 63 and from the same section. Judged newly formed lymph vessels are indicated by "a" and "b." Neither blood nor lymph vessels are present in the base of the portion of the pedicle in this section. Both must have reached the implant through the broad fused portion of the pedicle shown in Fig. 66. The continuity between vessels "a" and "b" of this photomicrograph with the lymph vessels shown in Fig. 66 cannot be established.  $\times 130$ .

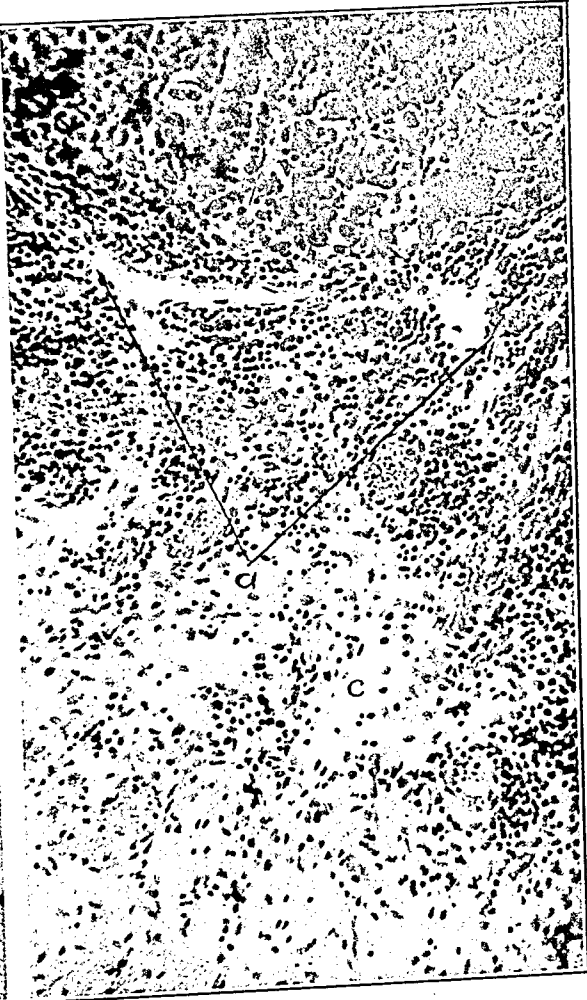
FIG. 68. Higher magnification of a judged lymph vessel "a" below the advancing carcinoma in Fig. 66 and indicated by "a" in that photomicrograph. Another judged lymph vessel is indicated by "c" in both photomicrographs.  $\times 130$ .



66



67



68

Sampson

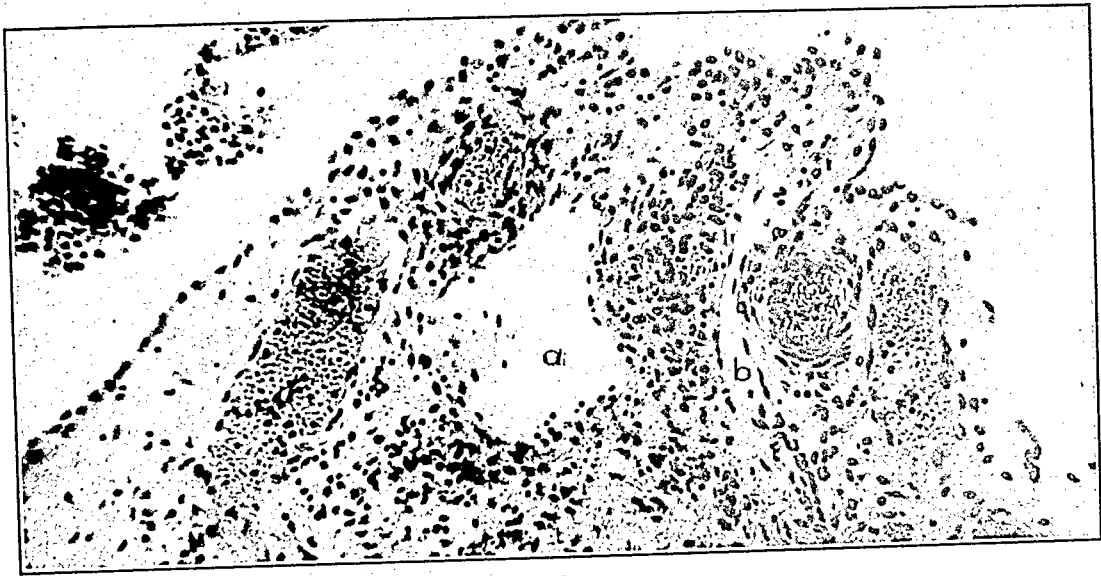
Lymph Vessels in Carcinomatous Peritoneal Implants

FIG. 69. A portion of the base of the pedicle of the implant shown in "D" of Fig. 63. Judged lymph vessels are indicated by "a" and "b." They are situated near blood vessels which are extending into the pedicle. The lymph vessel "a" shows that variation in the appearance of the oval flattened nuclei of its endothelial cells depends on the plane of section. A few lymphocytes are present in its lumen.  $\times 130$ .

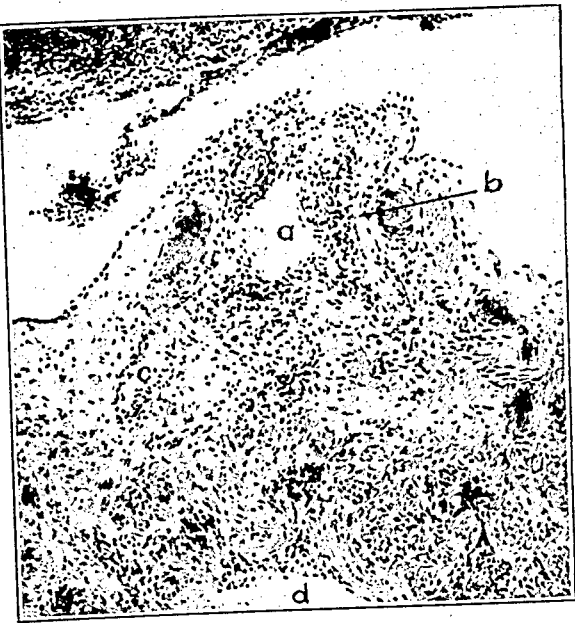
FIG. 70. Lower magnification of the base of the pedicle shown in Fig. 69, from the same section and better showing its relation to the implant above as well as to the peritoneum beneath it. The continuity of lymph vessel "a" with preëxisting lymph vessels is strongly suggested in the series of sections but is not definitely determined: "c" and "d" are lymph vessels in the sub-peritoneal tissues.  $\times 54$ .

FIG. 71. A section through the entire length of the pedicle of the implant shown in "D" of Fig. 63, a portion of the base of which is shown in Fig. 70. Lymph vessel "a" situated to the left of a mass of lymphocytes is a continuation of lymph vessel "a" in Fig. 70 and may be followed through all the intervening sections. It appears lower in the pedicle than in Fig. 70. This is due to the way in which the two prints are trimmed. In reality it is at about the same level. Its farther extension into the pedicle of the implant is suggested in this and other sections but cannot definitely be determined.  $\times 54$ .

FIG. 72. A section through the pedicle of the implant shown in Fig. 71. The level of this section is beyond the latter in the series. Lymph vessel "a" in Figs. 70 and 71 had disappeared in sections in the series previous to this one. A lymph vessel "a" is shown at the junction of the pedicle with the base of the main implant. Its continuity with lymph vessel "a" of Fig. 71 is not established. However, they may well be continuous without obvious evidence of this fact in non-injected vessels. The strand of granulation tissue to the right, also shown in "D" of Fig. 63, is attached to the peritoneum near the base of the pedicle of the larger portion of the implant, as shown in other sections of the series. The tissue in this strand is much younger than the stroma of the large implant. Although lymph vessels cannot be detected in it they may be present. Judged lymph vessels are found not only in the many pedicles of this implant but also in that portion of its stroma situated between the pedicles and the advancing carcinoma. Lymph vessels cannot be detected in the mass of carcinoma.  $\times 54$ .



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Sampson

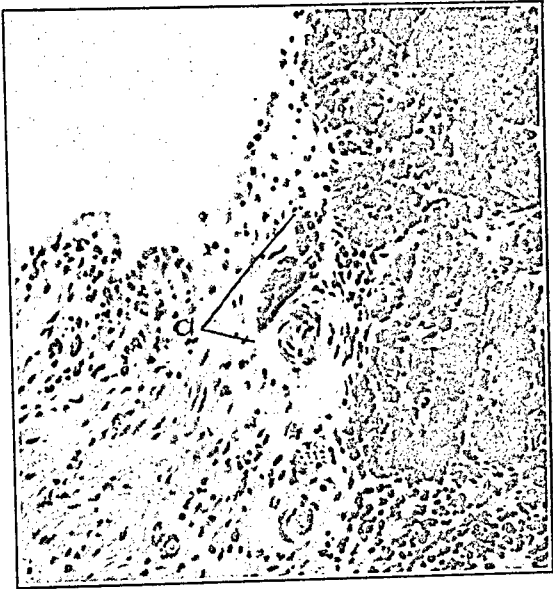
Lymph Vessels in Carcinomatous Peritoneal Implants

PLATE 75

- FIG. 73. A section showing the invasion of a judged preëxisting lymph vessel of the peritoneum by carcinoma from the implant shown in Fig. 42. By continuous extension the growth has penetrated the walls of this vessel at "a." For the farther extension of the carcinoma in the lumen of the lymphatic see the next two photomicrographs.  $\times 130$ .
- FIG. 74. A section near the preceding one showing the carcinoma in a definite lymph vessel "a." The carcinoma in this situation apparently is a continuation of that indicated by "a" of Fig. 73 and does not come from the portion of the growth nearest to it.  $\times 130$ .
- FIG. 75. A section showing the continuous permeation of the lymph vessel "a" by the carcinoma shown in "a" of Fig. 74. This section is situated in the series at some distance from that shown in Fig. 74. Carcinoma in this lymph vessel is present in all the intervening sections.  $\times 130$ .
- FIG. 76. A section very near but beyond the preceding one showing the lymph vessel "a" without carcinoma in it.  $\times 130$ .
- FIG. 77. Photomicrograph showing embolic carcinoma in a lymph vessel of the subperitoneal tissue to the left of the implant shown in the preceding illustrations. The carcinoma in this vessel may well have reached its present situation from the invasion of the lymph vessel just shown or from that of another but similar vessel. Carcinoma in an implant probably possesses potentialities of lymphatic permeation and metastasis similar to those of a primary growth.  $\times 130$ .



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Lymph Vessels in Carcinomatous Peritoneal Implants

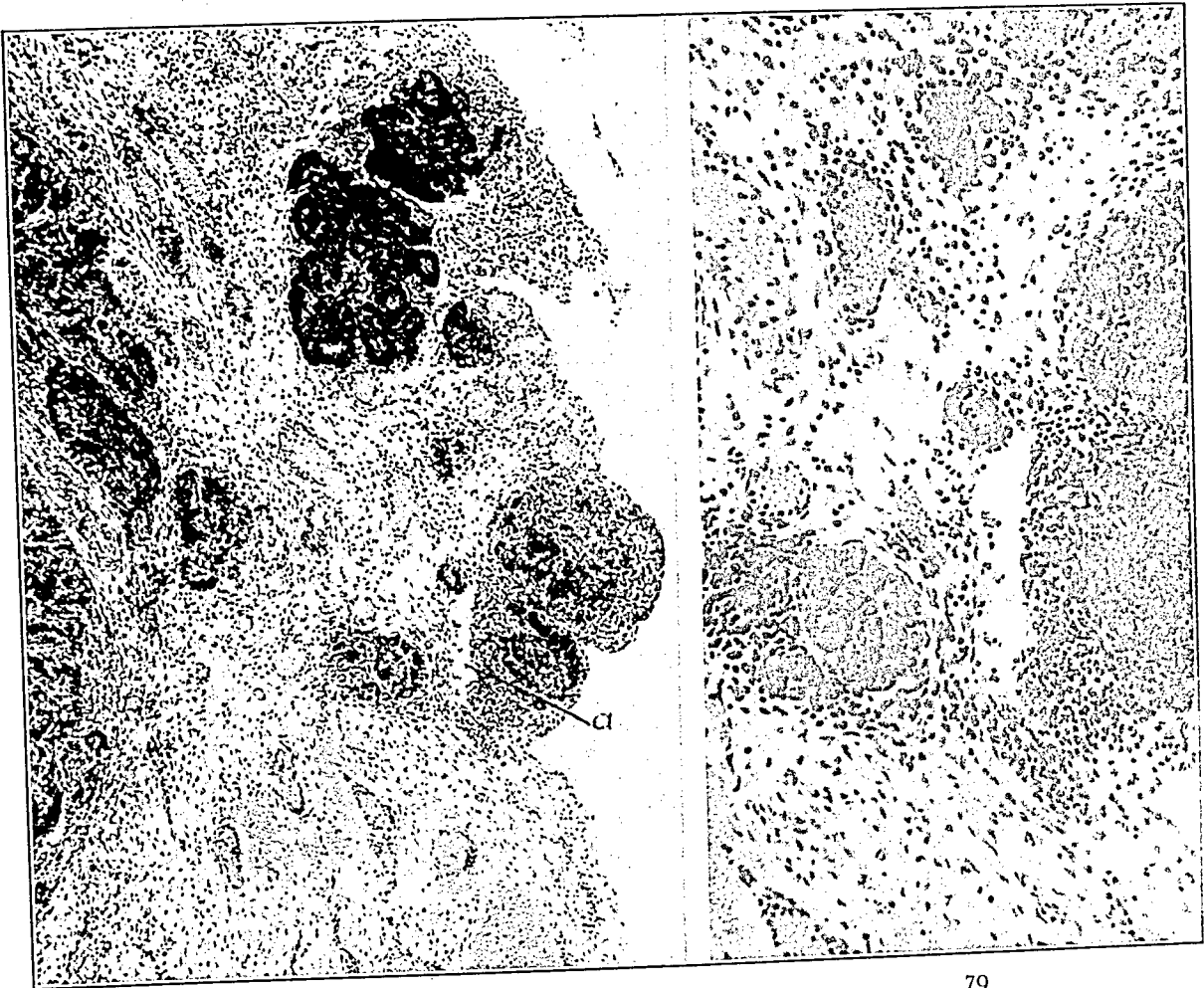
FIG. 78. Newly formed tissue at the right of the implant shown in Fig. 42, from a nearby section. Carcinoma is embedded in this loose vascular granulation tissue, the kind of tissue in which one would expect to find newly formed lymph as well as newly formed blood vessels. A portion of the main implant appears in the photomicrograph, at the left. A possible lymph vessel is indicated by "a." It is impossible to establish its continuity with preëxisting lymph vessels in the peritoneum beneath it: none can be detected in this portion of the peritoneum.  $\times 54$ .

FIG. 79. Higher magnification of the possible lymph vessel indicated by "a" of Fig. 78. It is difficult to say positively that it is a lymph vessel even though lymphocytes are present in its lumen. Carcinoma is apparently invading the upper portion of this space or vessel but, as shown in nearby sections of this series, it does not actually gain access to the lumen which quickly becomes obliterated by the pressure of the surrounding growth.  $\times 130$ .

FIG. 80. A portion of the base of the sessile polypoid implant and underlying peritoneum shown in Fig. 42, from another section. The implant has a fenestrated base due to granulation tissue arching over an intact portion of the mesothelial covering the peritoneum. The space thus created has become lined by mesothelium and is partially filled with a papillary mass of carcinoma. This mass may have arisen either as an outgrowth from the carcinoma at the left or as an implantation. The lower margin of this space represents the original level of the surface of the peritoneum. All structures above this level are newly formed. A dagger-shaped space is indicated by "a." This well may be a lymph vessel. The rounded handle of the dagger is probably a preëxisting vessel but the blade-like channel extending upwards into the implant is newly formed. The latter is surrounded by carcinoma which in places has apparently penetrated its lumen. The arrow points to a cell undergoing mitosis, in the lumen of this channel which is shown more highly magnified in the next photomicrograph.  $\times 130$ .

FIG. 81. Higher magnification of the channel or newly formed lymph vessel extending into the implant shown in Fig. 80. This vessel is surrounded by carcinoma which in places may have penetrated its walls. The arrow points to a cell undergoing mitosis. That it is a cancer cell cannot be proved even though it resembles unquestioned cancer cells which are actively dividing. If this is a cancer cell in a newly formed lymph vessel it demonstrates the part played by newly formed lymph vessels in the dissemination of cancer into the lymphatic circulation. Newly formed lymph vessels cannot be detected in the dense carcinomatous growth of the main implant.  $\times 325$ .





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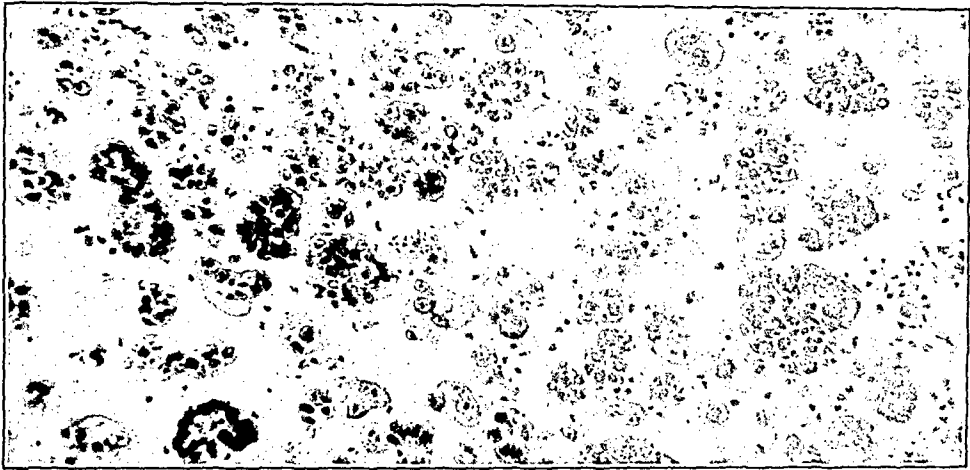
Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

FIG. 82. Sediment from the centrifugalized ascitic fluid of a patient with carcinoma of both ovaries with an associated peritoneal carcinomatosis (Case 5). Note the clumps of viable appearing cancer cells. These are found not only in the peritoneal cavity but also in the lumina of both tubes and in the lymph vessels of the ovaries and of the tubal and uterine walls. The cell arrangement in all of these situations is the same and is identical with that found in the judged primary ovarian tumors and in the peritoneal metastases (implantations). Is retrograde embolic metastasis from the ovarian tumors by way of the lymph stream the only possible explanation for the presence of cancer cells in the lymphatics of the tubal and uterine walls?  $\times 130$ .

FIG. 83. A cross-section of an epiploic appendage (Case 5) showing various types of peritoneal implants and various stages in their life history from the early fixation of clumps of cancer cells on the surface of the peritoneum to the fully organized implant. Lymphatics are not detected in any of these implants nor are they seen in the tissues of many sections of this epiploic appendage. The strongest possible circumstantial evidence indicates that these metastases arise from the implantation of cancer cells on the peritoneal surface of the epiploic appendage.  $\times 10$ .

FIG. 84. Four polypoid metastases on the peritoneal surface of the mesosalpinx. These are exact duplicates, more highly magnified, of implants shown on the surface of the epiploic appendage in Fig. 83, and from the same patient. Circumstantial evidence indicates that their pathogenesis is similar to that of the preceding ones. Lymph vessels as such cannot be detected in any of these implants. However, small clumps of cancer cells are present in judged lymphatics in the peritoneum beneath the attachment of the pedicle of the largest implant of the mesosalpinx. Strands of cancer cells are present in the pedicle of the implant, apparently extending towards the mesosalpinx beneath it. Because of the thickness of the section and the torn pedicle it is impossible to determine whether or not these strands of cancer cells are in lymph vessels. The circumstantial evidence just presented would lead to the conclusion that the carcinoma in the judged lymphatics beneath the pedicle of the implant comes from the implant itself and not from the ovarian tumors by retrograde metastasis.  $\times 25$ .



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Lymph Vessels in Carcinomatous Peritoneal Implants

# PLATE 78

FIG. 85. A cross-section of the Fallopian tube (Case 5) showing several polypoid implants on its peritoneal surface and cancer cells distending the lymph vessels of the tubal wall. The largest implant has several pedicles, two of which appear in this section. From the smaller pedicle, on the right, a broken line of clumps of cancer cells can be seen extending from the implant through the outer portion of the tubal wall to the lymph vessel showing the greatest distention with cancer cells (see the next illustration). What is the relation between the carcinoma in the implant on the peritoneum and that in the lymphatics? Carcinoma in the lymphatics of the tube in this section is found only in the vessels situated beneath the implant. Since the implants are duplicates of those shown in Figs. 83 and 84 one might infer that their pathogenesis was the same. A vein distended with blood is indicated by "v."  $\times 10$ .

FIG. 86. Higher magnification of the broken line of clumps of cancer cells extending from the smaller pedicle of the largest implant shown in Fig. 85 to the distended lymph vessel in the tubal wall beneath it. The upper portion of the lymph vessel distended with carcinoma appears at the bottom of the photomicrograph. One may follow the broken line of cancer cells through the pedicle of the implant and the outer portion of the tubal wall down to this lymph vessel. I believe that the line of cancer cells is in a lymph vessel which is a branch or tributary of the distended lymphatic in the tubal wall. This latter point cannot be determined positively. However, this section and those before and after it in the series strongly suggest this possibility. If the line of cancer cells in the outer but preëxisting portion of the tubal wall is actually in a lymph vessel one might infer that the similar appearing line of cancer cells in the pedicle of the implant and apparently continuous with that of the tubal wall is also in a lymph vessel. If so, the portion of the lymph vessel in the pedicle must be newly formed.  $\times 54$ .



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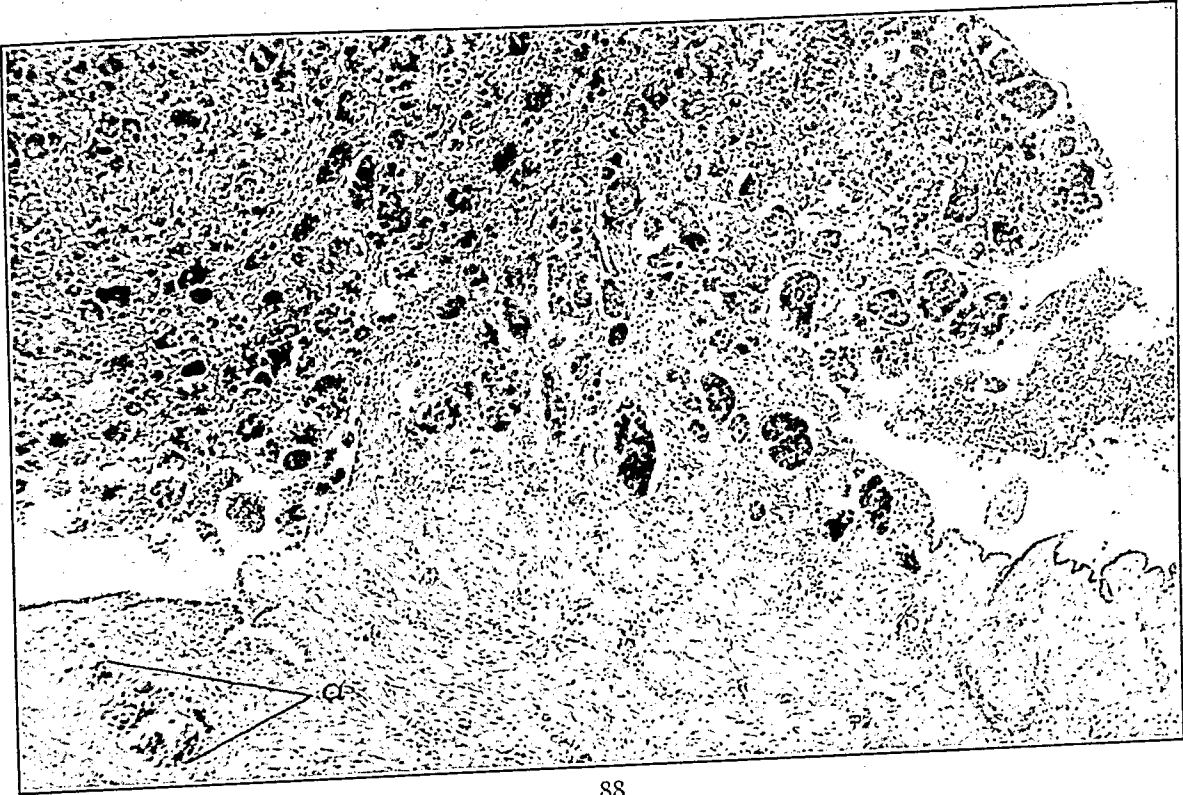
PLATE 79

FIG. 87. Higher magnification of the outer portion of the broader pedicle of the largest implant shown in Fig. 85. Broken lines of cancer cells are invading the tubal wall from the carcinoma above. It is impossible to ascertain whether or not these cancer cells are in lymph vessels. At the outer margin of the base of the pedicle one sees clumps of cancer cells in spaces which closely resemble dilated small lymph vessels. The lowest one of these may be a preëxisting lymph vessel. If so, the ones above it may be dilated, newly formed lymph vessels.  $\times 54$ .

FIG. 88. The same pedicle shown in Fig. 87, from another section. The histological picture suggests that the carcinoma of the implant is extending through its pedicle and invading the wall of the tube. In the base of the pedicle many of the spaces containing carcinoma may well be lymph vessels. If so, these would furnish channels for the spread of the carcinoma from the implant to the lymphatic circulation of the tube. Carcinoma is present in the tubal wall at "a." It is impossible to determine whether or not it reached its present situation from the nearby implant.  $\times 54$ .



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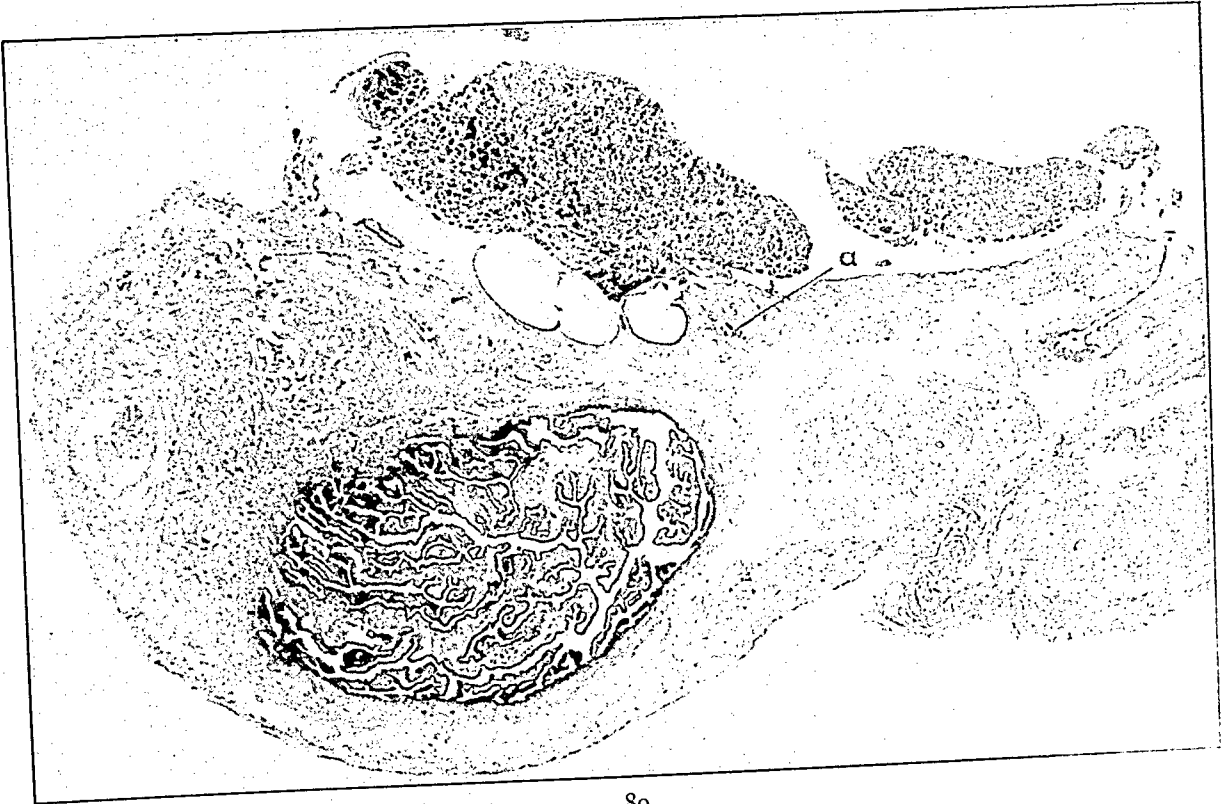
Lymph Vessels in Carcinomatous Peritoneal Implants

FIG. 89. A cross-section of the Fallopian tube and a portion of the mesosalpinx, showing carcinomatous implants on the surface of both (Case 5). Note that these implants are attached by slender pedicles to the surfaces of the wall of the tube and the mesosalpinx. These implants are mature and well may represent a later stage of the condition shown in Fig. 23. Carcinoma is found in the lymph vessels of the tubal wall in this section, but only in the portion of the wall beneath these implants (see Fig. 92).  $\times 10$ .

FIG. 90. Higher magnification of the slender, tortuous pedicle probably uniting the left pole of the larger implant of Fig. 89 to the surface of the tube. I believe that the broken line of clumps of cancer cells in the portion of the pedicle attached to the tube is situated in a lymph vessel. Since the pedicle arose from an outgrowth of the tissues of the tubal wall during the granulation tissue stage of the implant, all of the structures in it including the blood and lymph vessels must be newly formed. The structure at the right of the illustration may be a continuation of the pedicle. This point was not determined as serial sections were not made of this block. Lymph vessels are not detected in the scanty and dense stroma of the implant.  $\times 54$ .

FIG. 91. Higher magnification of the slender tortuous vascular pedicle apparently uniting the right pole of the smaller implant shown in Fig. 89 to the surface of the mesosalpinx. Note that the lower portion of the pedicle contains a large blood vessel, cut obliquely, and a clump of cancer cells in a space which histologically resembles a lymph vessel. The clumps of cancer cells in the tortuous portion of the pedicle above it may also be in lymph vessels. Lymph vessels may be found in pedicles similar to those shown here (see Figs. 23 and 25). Since serial sections were not made it is not possible to state that the pedicles shown in this illustration are portions of the same structure. The conditions seen in these pedicles may represent the attempted escape of carcinoma from the implant above them, through newly formed lymphatics of the pedicles. This pedicle is apparently younger than the one shown in Fig. 90.  $\times 100$ .

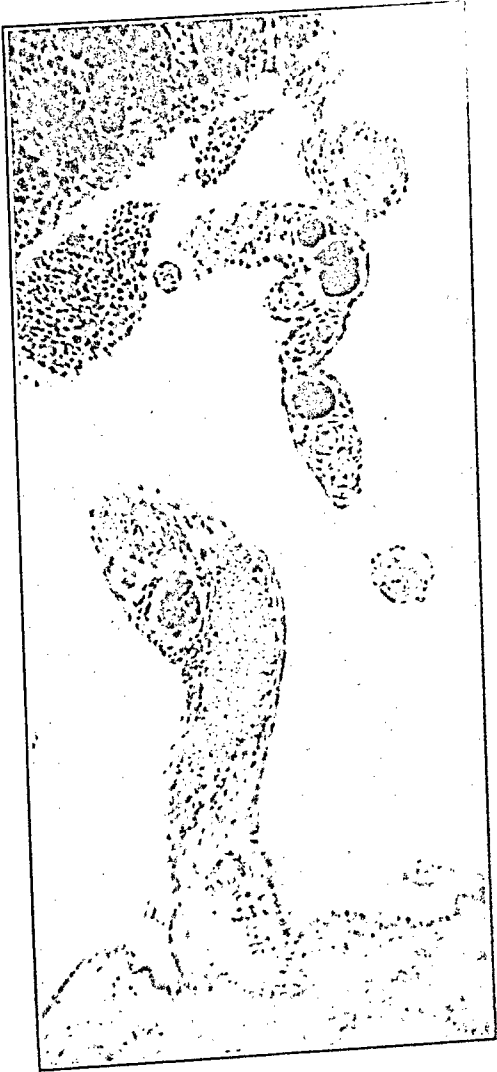




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Sampson

Lymph Vessels in Carcinomatous Peritoneal Implants

PLATE 81

FIG. 92. Higher magnification of a lymph vessel situated in the tubal wall beneath the base of a pedicle of the larger implant shown in Fig. 89 (see "a" of that illustration). The vessel contains clumps of cancer cells which must have reached their present situation either by having been conveyed in a retrograde manner through lymph vessels from the primary ovarian tumor, or by having been carried downstream from the carcinoma in the nearby implant through lymph vessels in its pedicle.  $\times 130$ .

FIG. 93. A portion of the base of a pedunculated polypoid implant on the surface of the Fallopian tube. This section is similar to those just shown and comes from the same patient. A cross-section of a mature tortuous pedicle appears at the left and presents a clump of cancer cells in a possible lymph vessel "a" (see also Fig. 94). The implant is mature. Its stroma for the most part is dense and contains very few blood vessels although in the peripheral portion of the tumor it is less dense and more vascular. Occasionally one finds in this portion of the implant cancer cells contained in a space "b" which very closely resembles the lumen of a lymph vessel.  $\times 54$ .

FIG. 94. Higher magnification of the cross-section of the pedicle shown in Fig. 93. I believe that the cancer cells may be implanted on the endothelial lining of a newly formed lymph vessel which accompanies the newly formed blood vessels of the pedicle. If lymph vessels are in the pedicle they also may be in the implant itself. Note the marked proliferation of mesothelial cells on the surface of the pedicle and their encapsulation of cancer cells. This represents one type of early implantation of carcinoma on the peritoneum.  $\times 130$ .

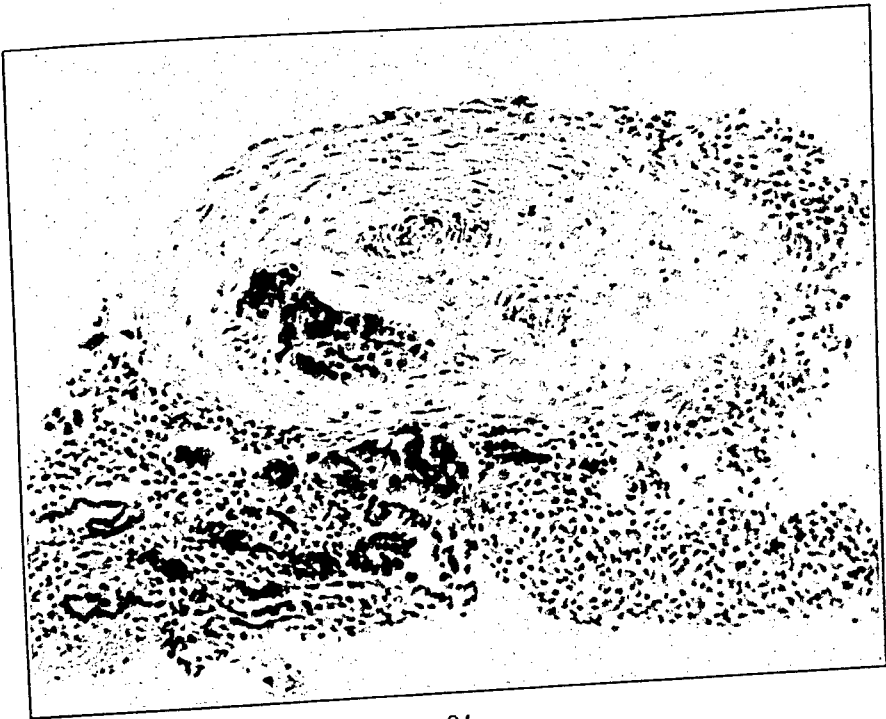
FIG. 95. A portion of the tubal wall beneath the implant shown in Fig. 93 and from the same section. Because this section is an old one and poorly stained it is difficult to make out clearly the cellular lining of the space in which the clumps of cancer cells are situated. I believe that they may be in a lymph vessel.  $\times 130$ .



92



93



94

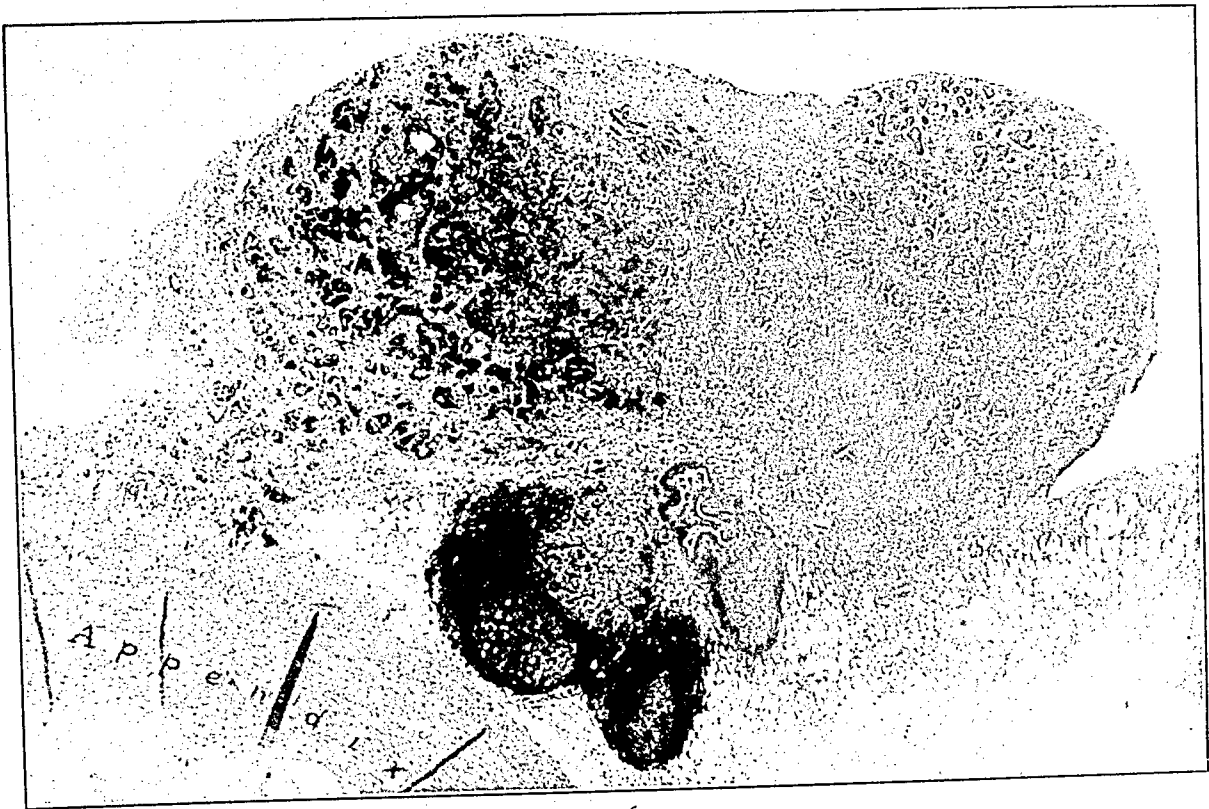


95

FIG. 96. A sessile polypoid implant on the mesoappendix at its attachment to the appendix, from a patient (Case 6) with carcinoma of both ovaries associated with peritoneal carcinomatosis. The tissues of the mesoappendix beneath the metastasis are invaded by the carcinoma. Of particular interest is the response of the serosa of the appendix to the carcinoma (see the next illustration).  $\times 25$ .

FIG. 97. Higher magnification of the surface of the appendix at its contact with the advancing edge of the carcinoma, from the section shown in Fig. 96. Adjacent to the invading carcinoma granulation tissue has developed on the surface of the appendix. Present in this tissue are dilated blood vessels some of which must be newly formed. These are accompanied by lymph vessels "a," "b" and "c" which are not only dilated but appear to be pushing out into the granulation tissue above them. A judged lymph vessel situated above vessel "b" and possibly continuous with the latter can be seen in a nearby section. Carcinoma is also present in this vessel. It is not known whether or not other lymph vessels extend into the adjacent and more adult newly formed tissue, above and to the right, which is invaded by the carcinoma. Lymph vessels as such cannot be recognized in this situation.  $\times 130$ .

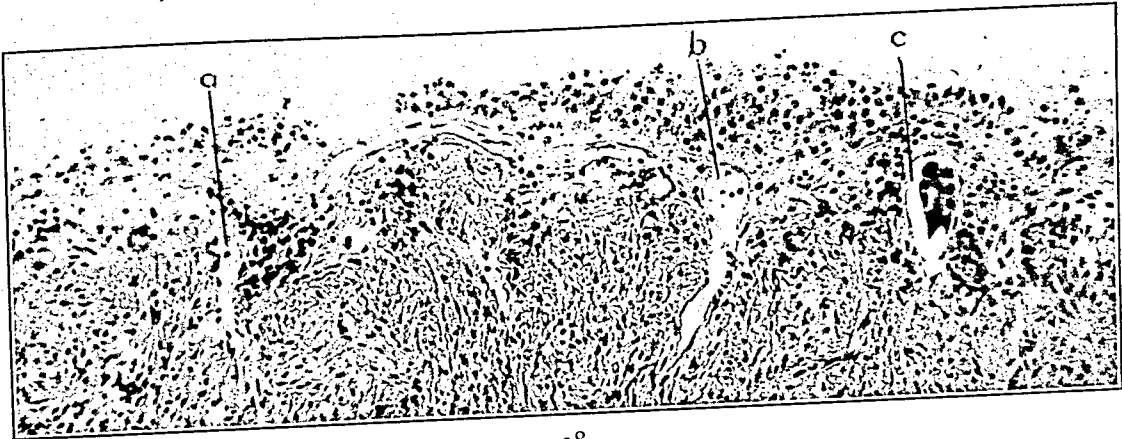
FIG. 98. The serosa of the appendix at a point close to that shown in Fig. 97. A peritoneal reaction is present which is characterized by a deposit of fibrin on its surface and the early formation of new tissue. This reaction becomes more pronounced as one approaches the carcinoma, to the right. The tips of judged lymph vessels "a," "b" and "c" in the muscularis are dilated and appear to be pushing outwards towards the new tissue above them. Carcinoma is present in one of these vessels, "c." The extent of their dilatation, bulging or apparent outgrowth increases as they approach the area shown in Fig. 97. Compare the lymph vessels shown in these two photomicrographs, using the outer margin of the muscularis of the appendix as a guide. In the preceding photomicrograph the portions of the vessels which can be seen show greater dilatation and are situated above the muscularis in granulation tissue which has replaced the serosa. The response of these vessels to the stimulation of carcinoma is believed to be the same as that of blood vessels. Both types of vessels play a part in the formation of the granulation tissue in this instance. The presence of dilated lymph vessels in this tissue, however, is of special significance, for they may permit an early dissemination of the nearby and invading carcinoma into the lymphatic circulation. Therefore, the reaction of the lymph vessels in this situation is not only of scientific interest but it is also of clinical importance.  $\times 130$ .



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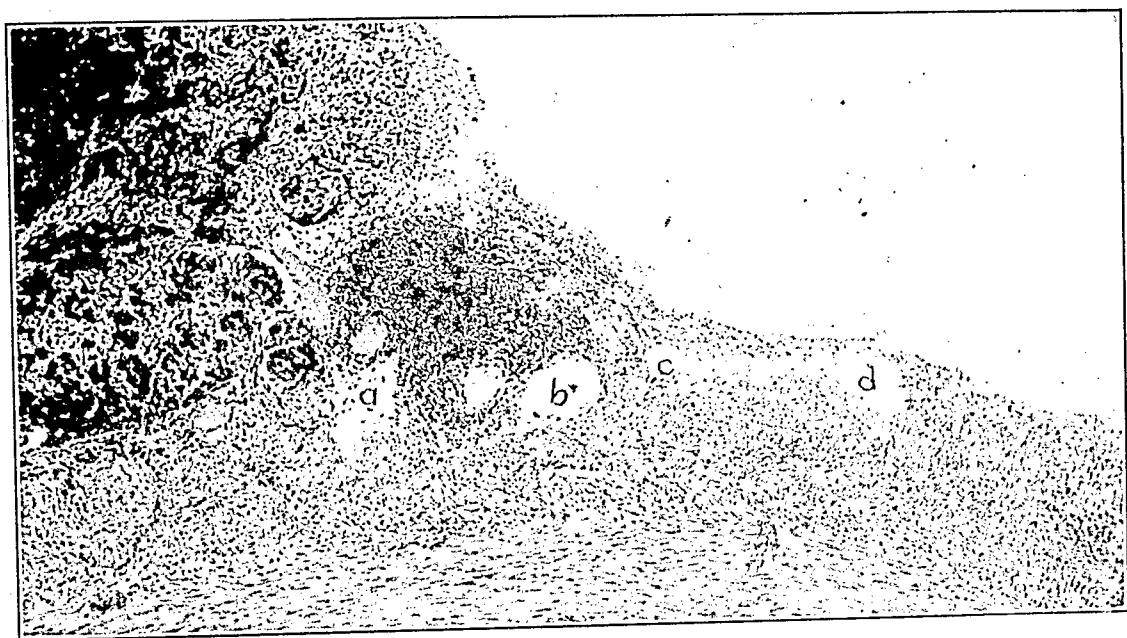


98

FIG. 99. The advancing edge of the carcinoma in an implant on the meso-appendix, similar to the one just described and from the same patient. Note the reaction of the serosa of the mesoappendix to the advancing carcinoma. The blood vessels are injected. The lymph vessels "a," "b," "c" and "d" are dilated. The response of these vessels is quite similar to that of the vessels shown in Fig. 97 except that here the surface of the peritoneum is still intact while in the other situation the serosa has been replaced by granulation tissue.  $\times 54$ .

FIG. 100. A section from the same block of tissue as Fig. 99 and very near it as is evident by comparing the two photomicrographs. Carcinoma is present in a dilated lymph vessel, presumably "a" of the preceding illustration. The tumor may well have reached this situation from the invasion of lymph vessels, which are continuous with vessel "a," by the carcinoma shown just above it and to the left in the two photomicrographs. This portion of the advancing carcinoma suggests this possibility. It is impossible to follow the lymph vessels into the main tumor where, if present, they are compressed or filled by the carcinoma.  $\times 54$ .

FIG. 101. Granulation tissue on the surface of the appendix from a patient, A. H. No. 85668, with carcinoma of both ovaries associated with peritoneal carcinomatosis. Carcinomatous implants are present on the surface of this appendix. The carcinoma in some of these implants has deeply invaded the wall of the appendix (see Case 5 of previous paper<sup>1</sup>). In this photomicrograph granulation tissue is shown with a judged dilated lymph vessel "a" and "b" which contains lymphocytes in its lumen and accompanies the newly formed vessels. Carcinoma in granulation tissue like this would have a ready channel for its dissemination into the lymphatic circulation.  $\times 54$ .



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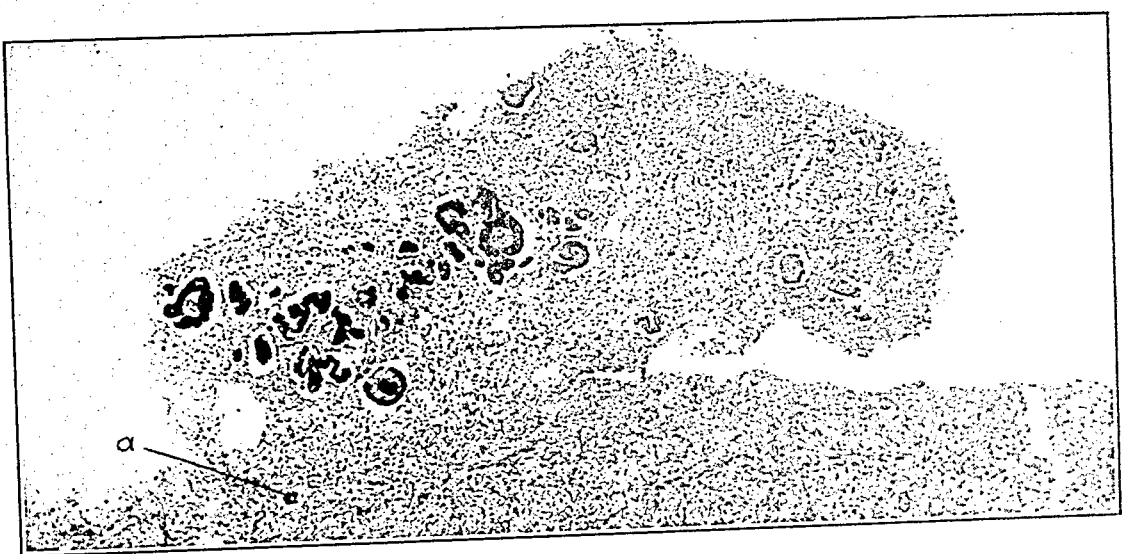
PLATE 84

FIG. 102. A small sessile polypoid implant in the early granulation tissue stage, on the surface of the ampulla of the Fallopian tube near its isthmus from a patient with carcinoma of both ovaries, associated with peritoneal carcinomatosis (Case 7). Even though it is evidently an early implant carcinoma is present in a lymph vessel "a" in the superficial portion of the muscularis. How may we explain the carcinoma in this situation other than by a retrograde metastasis or lymphatic permeation from the ovarian carcinoma? (See Figs. 103 to 113 inclusive.) What is the possible significance of the dilated spaces about some of the cancer cells in the implant? Are they all artefacts due to unequal tissue shrinkage? Could some of them be dilated newly formed lymph vessels which have been invaded by the carcinoma embedded in the tissues about them? (Compare with the above mentioned photomicrographs.)  $\times 25$ .

FIG. 103. Granulation tissue on the surface of the Fallopian tube adjacent to the implant shown in Fig. 102, from another section. The peritoneal reaction is greatest near the implant, to the left, just as it is in Fig. 97. Small cyst-like spaces which do not contain cancer cells are present in this granulation tissue. They are very similar to the dilated lymph vessels shown in Figs. 97, 99 and 101, as well as to some of the spaces containing carcinoma in Fig. 102.  $\times 54$ .

FIG. 104. Higher magnification of the thickest portion of the granulation tissue shown in Fig. 103 (compare with Fig. 97). This tissue has been traumatized during the operation, as shown by the extravasation of blood in it. Therefore, the identification of all of its elements is difficult. The structure indicated by "v" is possibly a dilated vein. It is filled with red blood corpuscles with a rim of leukocytes about them. The structure indicated by "a" is probably not a vein. It has an endothelium-like lining and contains blood and debris. The identity of "b" is also uncertain. Both of these structures may be lymph vessels. The extravasated blood may well have escaped into the lumina of these two vessels through their injured walls. For the probable origin of these vessels see the next four photomicrographs.  $\times 130$ .

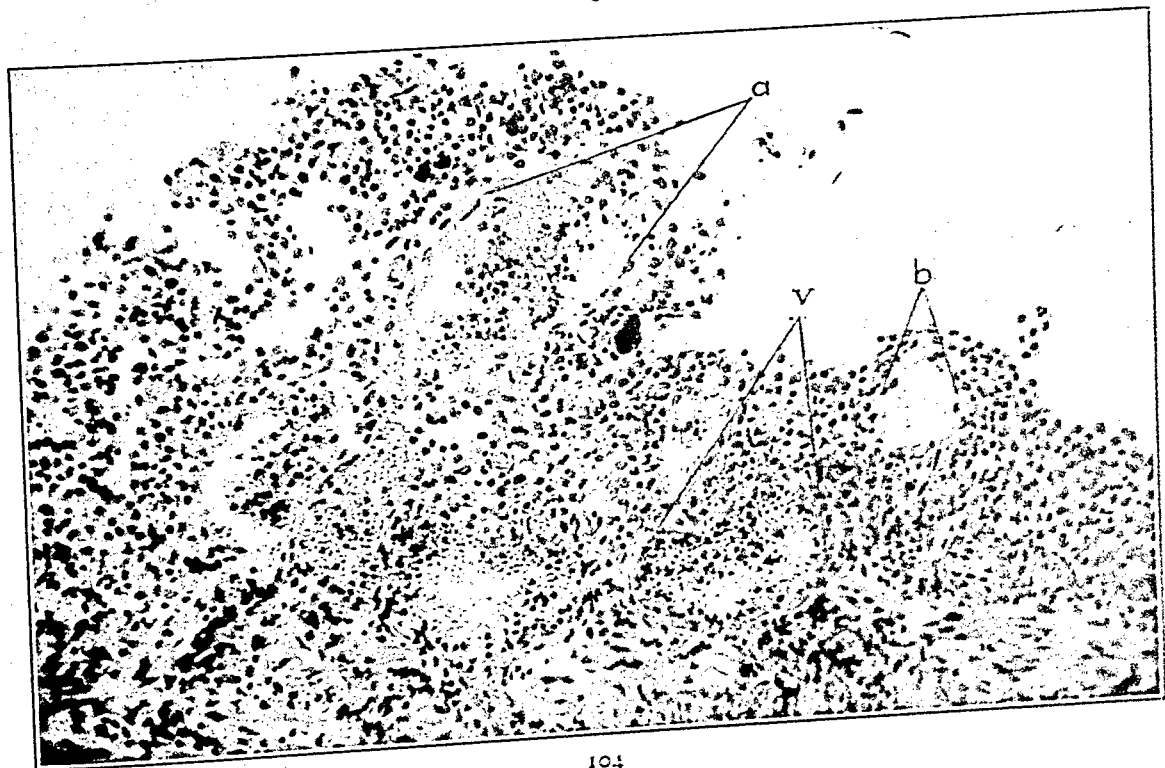




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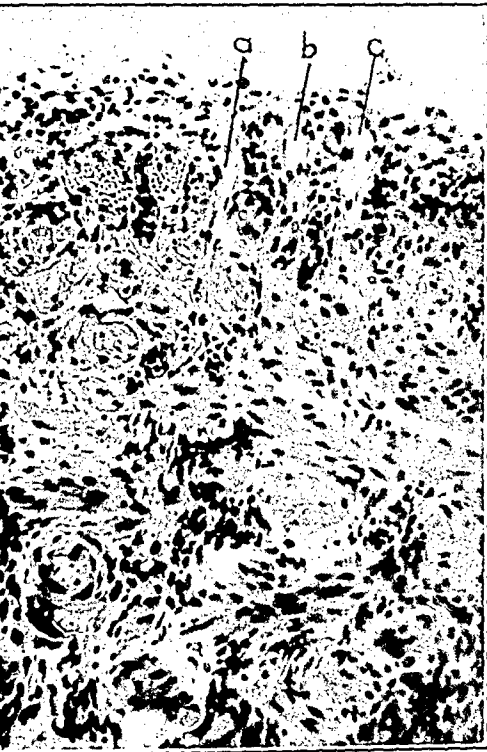
Lymph Vessels in Carcinomatous Peritoneal Implants

FIG. 105. A portion of the tubal wall farther away from the implant of Fig. 102 than is the peritoneal reaction shown in Fig. 103. There is here a peritoneal reaction similar to that shown in Fig. 98. As in the latter situation, the bulbous tips of what are believed to be lymph vessels "a," "b" and "c," are bulging or extending into the newly formed tissue on the surface of the Fallopian tube. These buttonhole-like vessels may be traced into the muscularis but not as deeply as those shown in Fig. 98.  $\times 130$ .

FIG. 106. A later stage of the same peritoneal reaction as that shown in Fig. 105, from another section. The serosa is replaced by early granulation tissue which is thicker than that shown in Fig. 105. The lymph vessels "a," "b" and "c" are more dilated than those in Fig. 105 and bulge or extend farther into the granulation tissue (compare with Figs. 97 and 98). It is not possible to identify the vessel indicated by "d-e" or the deeply staining mass of cells marked "x."  $\times 130$ .

FIG. 107. A later stage of the peritoneal reaction shown in Fig. 106. The possible lymph vessels are more dilated than those in Fig. 106 and have almost reached the surface of the granulation tissue. The origin of these structures from the lymph vessels of the muscularis is not as evident in this section as in the preceding two photomicrographs.  $\times 130$ .

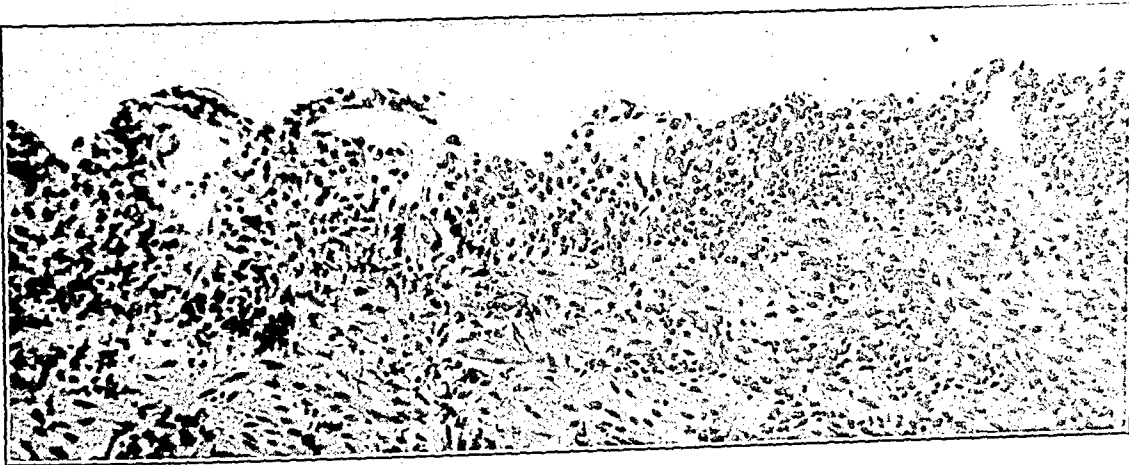
FIG. 108. A still later stage of the peritoneal reactions and the response of the lymph vessels shown in the three preceding photomicrographs. The granulation tissue is thicker and more vascular. The lymphatics are larger and resemble blebs in the section. Bleb "a" contains red blood corpuscles and cells which well may have escaped into its lumen from the extravasated blood in the tissues beneath it (compare with bleb "a" in Fig. 104). Bleb "b" is intact probably because of the fact that there is no extravasation of blood in the tissues about it. A dilated blood vessel is situated in the granulation tissue at its left (compare with Fig. 122). The continuation of these dilated lymph vessels into the underlying muscularis, as shown in Figs. 98 and 105, cannot be detected in this section but might have been seen in adjacent sections if the series had been complete. On the other hand, the lumina of these portions of the vessels may be occluded by the pressure of the denser tissues through which they pass (compare with Fig. 97). Such pressure may increase the dilatation of the portions of the lymph vessels in the relatively loose granulation tissue. It is also possible that the ostensible blebs "a" and "b" represent cross-sections of loops of dilated lymphatics and not sections of dilated bulbous tips of lymph vessels. Vessel "c" is a judged lymph vessel in the muscularis, which may be continuous with that in the granulation tissue above it. The study of these last four photomicrographs demonstrates the origin of some of the dilated spaces shown in Fig. 104 as well as similar structures shown in Figs. 102, 111 and 112.  $\times 130$ .



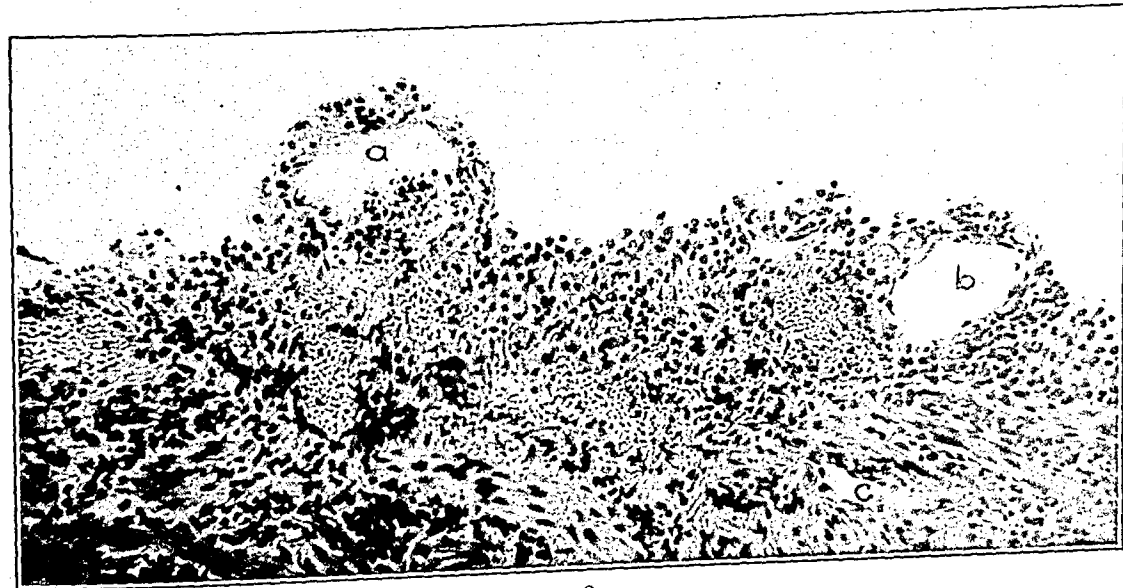
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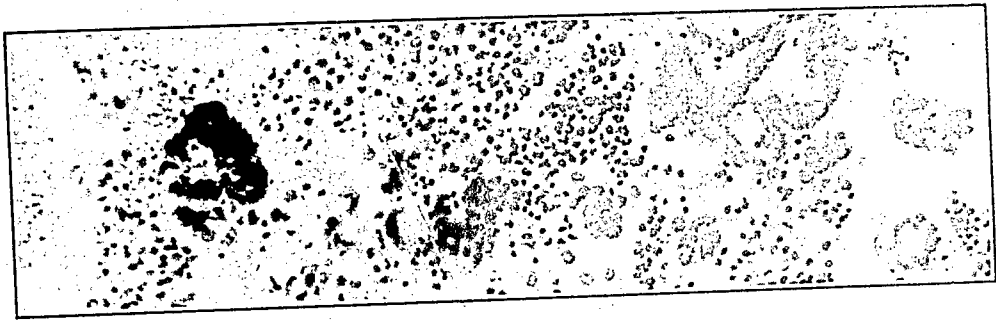


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108

- FIG. 109. A section of the sediment from the centrifugalized ascitic fluid obtained from the same patient (Case 7). Clumps of judged cancer cells are present in this sediment. Similar cancer cells often become implanted on the peritoneum. The metastasis shown in Fig. 102 represents a common phase in the development of such an implant. The peritoneal reactions, shown in Figs. 105 to 108 inclusive, as well as in Fig. 104, should have also been present in the early stages of the development of the implant shown in Fig. 102. The same or even a farther extension (growth) of the lymph vessels, similar to that shown in the preceding illustrations, should be present in the granulation tissue stroma of the implant. Therefore, at least some of the dilated spaces shown in Fig. 102, which apparently have been invaded by the neoplasm, may be newly formed lymph vessels.  $\times 130$ .
- FIG. 110. A portion of the implant shown in Fig. 102, from another section. Carcinoma enmeshed in granulation tissue is shown in the upper portion of the photomicrograph. A blood vessel is indicated by "b" and a possible lymph vessel accompanying it by "a." The lymph vessel contains a few lymphocytes and is possibly continuous with a similar channel, marked "c," just within the muscularis of the tube.  $\times 130$ .
- FIG. 111. Another portion of the implant, very near the one shown in Fig. 110 and the same distance above the muscularis, the outer border of which appears in the lower portion of both photomicrographs. A judged dilated newly formed lymph vessel surrounded by loose and possibly edematous tissue is indicated by "a." This vessel is lined by endothelium-like cells, the nuclei of which are unlike those of the fibroblasts about it. Its wall, at the left, has been torn, allowing the extravasated blood in the surrounding tissue to escape into its lumen. It accompanies a blood vessel which is present at the right. I believe that this structure represents a farther growth into the granulation tissue of a newly formed lymph vessel similar to those previously shown. Space "b" may well represent a continuation of this vessel into the muscularis (compare with Fig. 110). Clumps of cancer cells are situated above and to the right of vessel "a." One of these clumps, "c," is in a vessel which may well be an extension of "a." The sections in the series are too far apart to determine either the continuity of the lymph vessels or the actual penetration of their lumina by the carcinoma embedded in the tissues about them.  $\times 130$ .
- FIG. 112. A portion of the base of the implant shown in the preceding illustrations. Carcinoma is present in a judged lymph vessel which is situated just above the muscularis. This vessel well may be a dilated preëxisting structure and possibly is a continuation of the one in the muscularis to the right, indicated by "a." Carcinoma in newly formed lymph vessels similar to that shown in Fig. 111 may explain the presence of the neoplasm in the present situation.  $\times 130$ .
- FIG. 113. Higher magnification of the portion of the section shown in Fig. 102 in which carcinoma is present in a lymph vessel "a" in the superficial portion of the muscularis. I believe that the evidence just presented indicates that the invasion of newly formed lymph vessels in the stroma of the implant by carcinoma and its farther extension into preëxisting lymph vessels from which the vessels of the implant were derived, explains the presence of dissemination in the situation shown here better than any other mode of lymph vessels in carcinomatous implants on the peritoneum permit an earlier dissemination of the carcinoma into the lymphatic circulation than would otherwise arise.  $\times 130$ .



109



110



111



112



113

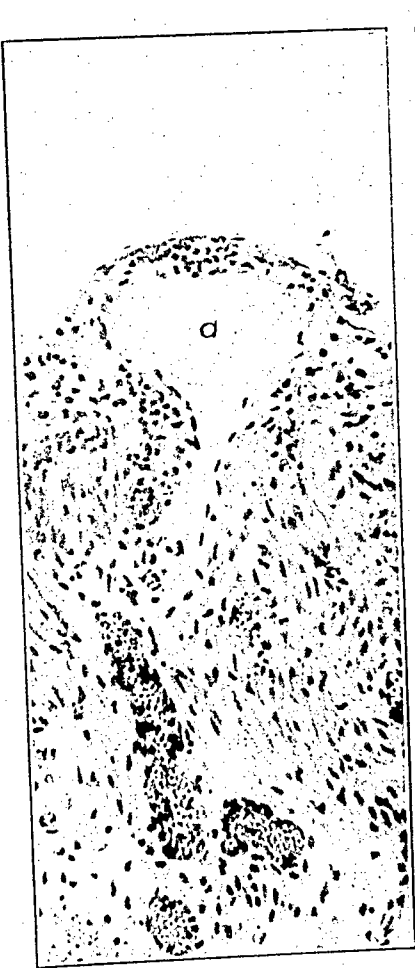
Lymph Vessels in Carcinomatous Peritoneal Implants

FIG. 114. A section of a bleb-like elevation on the surface of the peritoneum to the left of the large sessile polypoid implant shown in Fig. 52. There is evidence of a previous peritoneal reaction about it. A narrow prolongation of this bleb extends down into the peritoneum. This extension is accompanied by a blood vessel at its left. The entire structure very closely resembles the dilated lymph vessels in shallow granulation tissue on the surface of the peritoneum shown in Figs. 97 and 108.  $\times 130$ .

FIG. 115. A small isolated patch of granulation tissue situated on the surface of the peritoneum between the lymphatic bleb shown in Fig. 114 and the sessile polypoid implant mentioned in the legend of that illustration. The granulation tissue is capped by a large mass of exfoliated mesothelial cells. A cyst-like cavity is present in this granulation tissue. Is it a space created by the rapidly growing granulation tissue or a lymphatic bleb extending out into this tissue? This cyst-like cavity was followed through many sections in order to determine whether or not it was continuous with the lumen of a preëxisting lymph vessel (see the two following photomicrographs).  $\times 130$ .

FIG. 116. The same granulation tissue as that shown in Fig. 115. The cyst-like cavity "a" in this photomicrograph is a continuation of "a" in Fig. 115. A blood vessel "b.v." is situated to the right of it. The cellular lining of the cyst, below the letter "a," appears to be dipping into the base of the granulation tissue on the surface of the peritoneum. A meshwork of lymph vessels "lym." in the subperitoneal tissue is situated below this space. Their extension upwards towards the cyst is suggested in this photomicrograph but is much better shown in the colored photomicrograph Fig. 124 which is from a section following this one.  $\times 130$ .

FIG. 117. The same granulation tissue as that shown in Fig. 115, from a section beyond it in the series. The cyst in the granulation tissue has disappeared. The blood vessel, "b.v." in the granulation tissue is the same as that shown in Fig. 116 and is also a continuation of the vessel "b.v." in the subperitoneal tissue to the right of lymphatic "a." The latter is a continuation of the lymph vessels "lym." shown in Figs. 116 and 124. I realize that the continuity of the cyst-like structure in the granulation tissue with the preëxisting lymph vessels in the tissue beneath it has not been definitely established. However, strong circumstantial evidence has been presented to indicate that this continuity does exist but cannot be definitely established because of the obliteration of the lumen of portions of the lymph vessel by the pressure from surrounding tissues.  $\times 130$ .



114



115



116



117

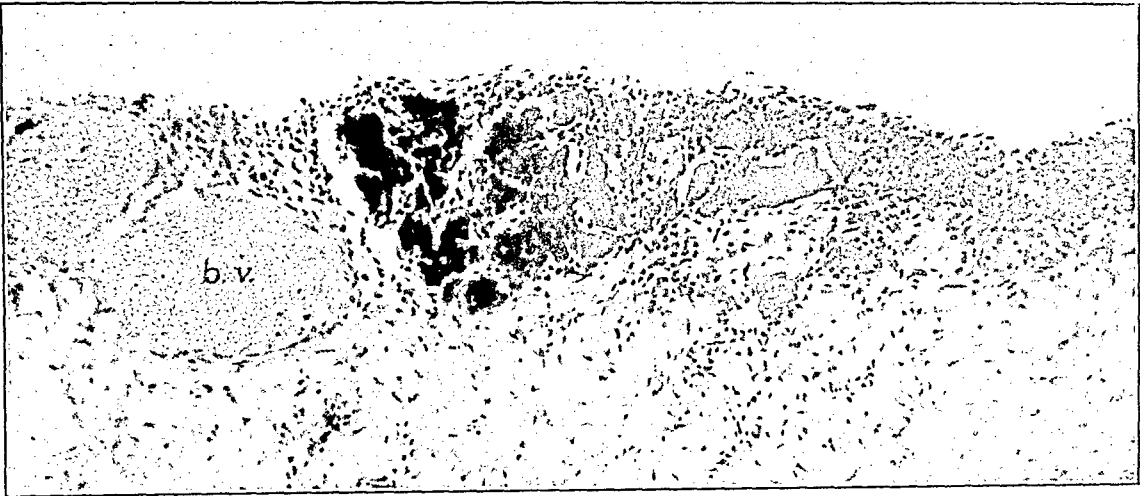
PLATE 88

FIG. 118. Parietal peritoneum with carcinoma implanted in it from a patient with carcinoma of both ovaries and an associated peritoneal carcinomatosis (Case 8). Small implantations like these are of frequent occurrence. Their pathogenesis has been fully described in Case 4 of a previous paper.<sup>1</sup> Newly formed tissue is present on the surface of the peritoneum containing dilated newly formed blood vessels "b.v." The condition suggests a chronic form of low granulation tissue which has failed to undergo involution because of the presence of carcinoma in it. If newly formed lymph vessels similar to those shown in Figs. 98, 107 and 108 were present in this tissue they might permit an earlier dissemination of the carcinoma into the lymphatic circulation. Since the implant is small and apparently insignificant it might easily be overlooked in the attempt to ascertain the original portal of entry of the carcinoma into the lymph vessels.  $\times 130$ .

FIG. 119. Another section of the implant shown in Fig. 118. It shows the extension of the carcinoma from the surface of the peritoneum into the underlying tissues. It is impossible to determine whether or not the carcinoma in the underlying tissues is in lymph vessels. It may be. Dilated newly formed lymph vessels in shallow granulation tissue, previously described, would furnish channels by which carcinoma implanted in that tissue could invade underlying structures.  $\times 130$ .

FIG. 120. A very mature encapsulated implant in the parietal peritoneum. It well may represent a later stage of the implant shown in Figs. 118 and 119. The section is from the same patient as these. Carcinoma is present in the tissues beneath the base of the implant. It appears as though it were extending from the implant into these tissues. Possibly it is situated in lymph vessels. Carcinoma is present in a judged lymph vessel indicated by "a." A small portion of a large sessile polypoid implant is shown at the left in the photomicrograph. Various types of implants on the parietal peritoneum of this patient were studied. Only a very few of them showed any invasion of the underlying lymph vessels. Many more implants on the peritoneum in other situations, from this patient, were also studied. In only one of these was carcinoma found in the lymph vessels beneath it. The carcinoma of the ovaries apparently arose from their surface epithelium. Carcinoma was not found in the lymph vessels of either ovary. There is thus the strongest possible evidence that the invasion of the peritoneal lymph vessels, in this patient, came from carcinoma implanted on the surface of the peritoneum.  $\times 54$ .

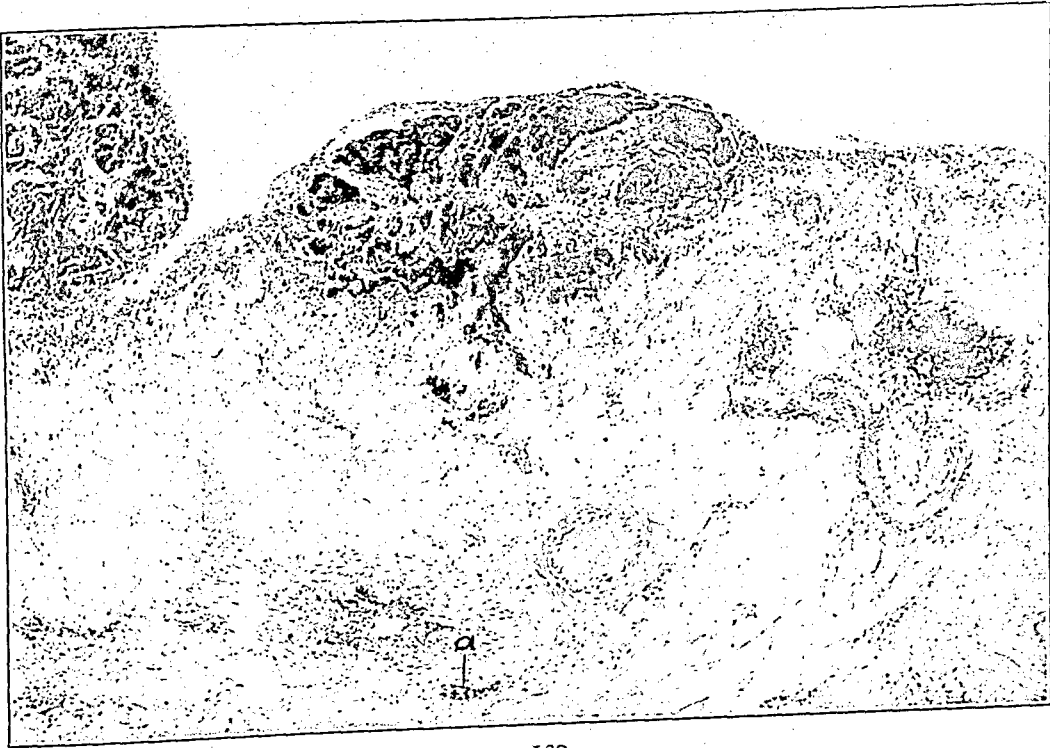




118



119



120

Lymph Vessels in Carcinomatous Peritoneal Implants

FIG. 118. Parietal peritoneum with carcinoma implanted in it from a patient with carcinoma of both ovaries and an associated peritoneal carcinomatosis (Case 8). Small implantations like these are of frequent occurrence. Their pathogenesis has been fully described in Case 4 of a previous paper.<sup>1</sup> Newly formed tissue is present on the surface of the peritoneum containing dilated newly formed blood vessels "b.v." The condition suggests a chronic form of low granulation tissue which has failed to undergo involution because of the presence of carcinoma in it. If newly formed lymph vessels similar to those shown in Figs. 98, 107 and 108 were present in this tissue they might permit an earlier dissemination of the carcinoma into the lymphatic circulation. Since the implant is small and apparently insignificant it might easily be overlooked in the attempt to ascertain the original portal of entry of the carcinoma into the lymph vessels.  $\times 130$ .

FIG. 119. Another section of the implant shown in Fig. 118. It shows the extension of the carcinoma from the surface of the peritoneum into the underlying tissues. It is impossible to determine whether or not the carcinoma in the underlying tissues is in lymph vessels. It may be. Dilated newly formed lymph vessels in shallow granulation tissue, previously described, would furnish channels by which carcinoma implanted in that tissue could invade underlying structures.  $\times 130$ .

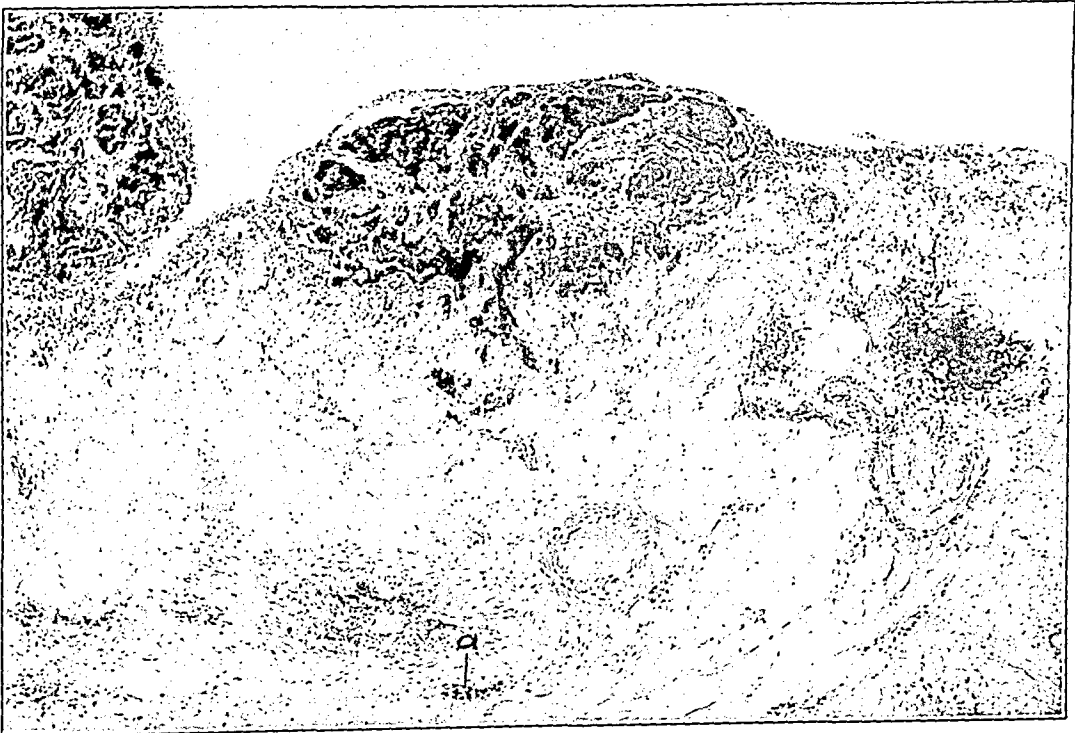
FIG. 120. A very mature encapsulated implant in the parietal peritoneum. It well may represent a later stage of the implant shown in Figs. 118 and 119. The section is from the same patient as these. Carcinoma is present in the tissues beneath the base of the implant. It appears as though it were extending from the implant into these tissues. Possibly it is situated in lymph vessels. Carcinoma is present in a judged lymph vessel indicated by "a." A small portion of a large sessile polypoid implant is shown at the left in the photomicrograph. Various types of implants on the parietal peritoneum of this patient were studied. Only a very few of them showed any invasion of the underlying lymph vessels. Many more implants on the peritoneum in other situations, from this patient, were also studied. In only one of these was carcinoma found in the lymph vessels beneath it. The carcinoma of the ovaries apparently arose from their surface epithelium. Carcinoma was not found in the lymph vessels of either ovary. There is thus the strongest possible evidence that the invasion of the peritoneal lymph vessels, in this patient, came from carcinoma implanted on the surface of the peritoneum.  $\times 54$ .



118



119



120

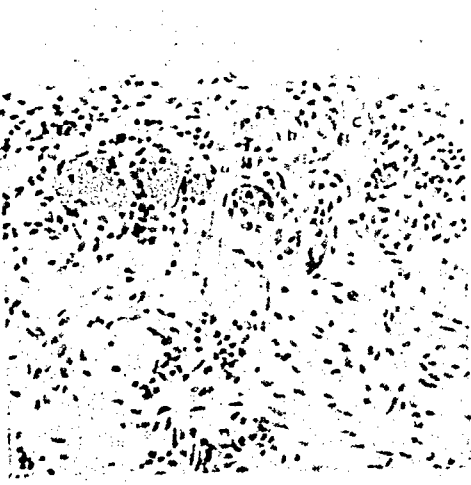
PLATE 89

FIG. 121. The same photomicrograph shown in Fig. 105 which has been colored in order to emphasize the part played by both blood and lymph vessels in a peritoneal reaction caused by cancer cells escaping into the peritoneal cavity. The judged lymph vessels "a," "b" and "c" have outstripped the blood vessels in their expansion or extension into the newly formed tissues on the surface of the Fallopian tube.  $\times 130$ .

FIG. 122. A portion of the photomicrograph shown in Fig. 108 (also colored) picturing a later stage of the peritoneal reaction shown in Fig. 121 (compare with Figs. 106, 107 and 108).  $\times 130$ .

FIG. 123. The photomicrograph, shown in Fig. 98, which has been colored to demonstrate better the progressive expansion or extension of the judged lymph vessels "a," "b" and "c" of the muscularis of the appendix into the newly formed tissue on its surface. Carcinoma is present in lymph vessel "c" (compare with Figs. 97 and 98).  $\times 130$ .

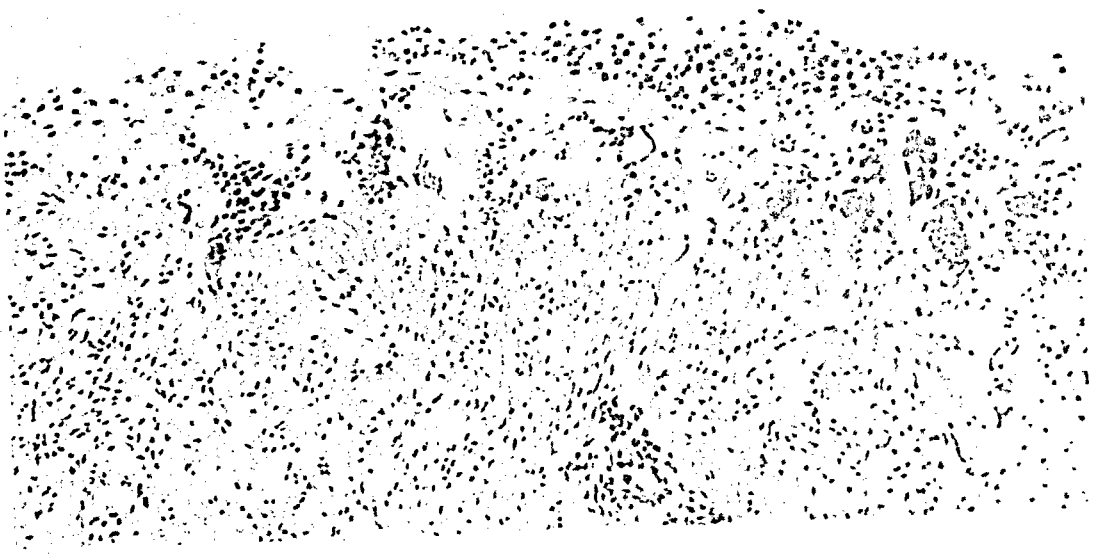
FIG. 124. Colored photomicrograph of a section adjacent to that shown in Fig. 116. The continuity between the preëxisting lymph vessel "lym." and the dilated, possibly newly formed lymph vessel "a" in the granulation tissue above it is better portrayed than in Fig. 116. Note the extension of the preëxisting lymph vessel towards (see arrow) and almost into the possibly newly formed lymphatic "a" which is adjacent to a newly formed blood vessel (compare with Figs. 115, 116 and 117).  $\times 130$ .



121



122



123



124

FIGS. 125 and 126. Carcinoma in the lymphatics of the wall of the left Fallopian tube from a patient, A. H. No. 9895, with carcinoma of both ovaries associated with peritoneal carcinomatosis. Extensive implantation metastases were found in the omentum and in both culs-de-sac. Implants of various types were also present in many other situations. However none was observed on the surface of either tube. The ovarian tumors were adherent to the posterior surface of the uterus and had invaded that organ, including the lymph vessels of the myometrium. Carcinoma is present in the lymph vessels of all portions of the left tube including its fimbriae and mesentery. In Fig. 125 the carcinoma is shown in the lymphatics beneath the tubal mucosa and in Fig. 126 in similar channels beneath the serosa. The histological study of this tube indicates that the distribution of the carcinoma in it is due to retrograde lymphatic permeation and metastasis. In places newly formed tissue is present on the serosa of the tube (see the following photomicrographs).  $\times 54$ .

FIG. 127. Newly formed tissue which has arched over the surface of the tube. This tissue contains newly formed blood vessels which are accompanied by newly formed lymph vessels. The origin of the latter from preëxisting lymphatics can be seen plainly in the two attachments of this newly formed tissue to the serosa of the tube, one of which appears in this photomicrograph, and the other in the next photomicrograph. Carcinoma is not present in this newly formed tissue which forms the stroma of many implants. If it had been present it might have invaded the nearby newly formed lymphatics and thus spread to the preëxisting lymphatics of the tube. If carcinoma had been present in the preëxisting lymph vessels beneath the attachment of the newly formed tissue it might have spread into the newly formed lymph vessels.  $\times 54$ .

FIG. 128. The other attachment of the newly formed tissue shown in Fig. 127 to the surface of the tube. Newly formed lymph vessels are also present in this tissue. The study of the series of intervening sections indicates that these vessels are continuous with those shown in Fig. 127.  $\times 54$ .

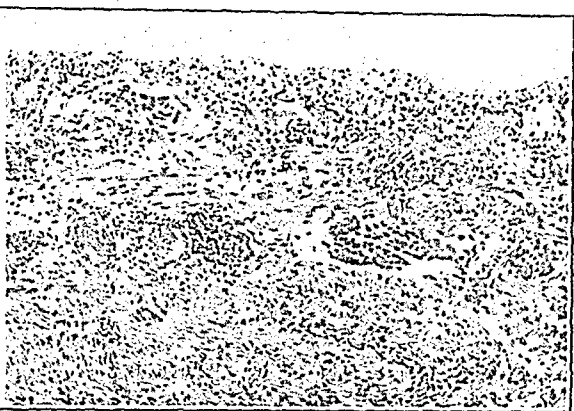
FIG. 129. A portion of the serosa and subserosa of the tube from the same series of sections shown in the preceding photomicrographs. Carcinoma is present in the lumen of a lymph vessel of the subserosa (see the next photomicrograph).  $\times 54$ .

FIG. 130. A field similar to the preceding one and near it in the series of sections. The same lymph vessel, shown in Fig. 129, has extended outwards into newly formed tissue which has arisen on the surface of the tube. This tissue forms one of the attachments of the arch of newly formed tissue to the surface of the tube shown in the next photomicrograph.  $\times 54$ .

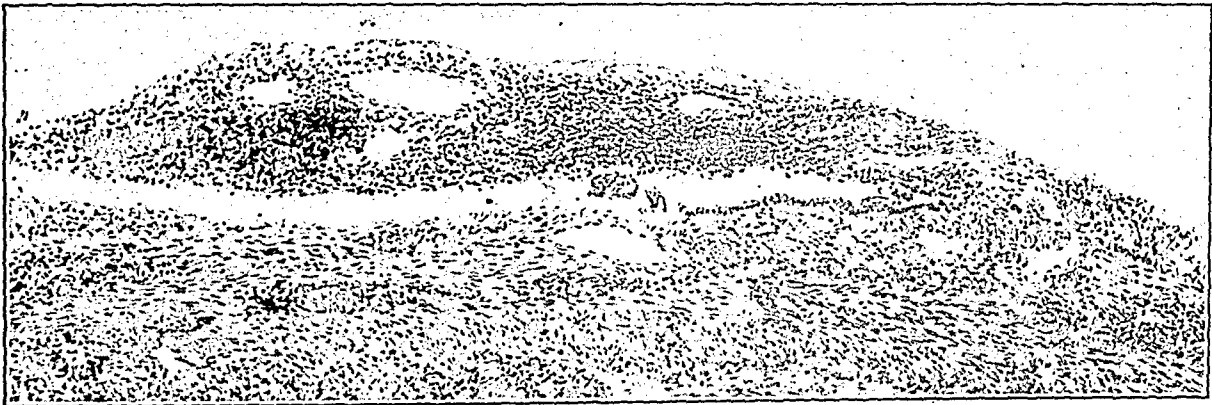
FIG. 131. Newly formed tissue which has arched over the surface of the tube. Another portion of the attachment of this tissue to the tube is shown in Fig. 130. This tissue contains a newly formed lymph vessel with carcinoma in its lumen. This vessel is shown in the series of sections to be continuous with the lymph vessel in the two preceding photomicrographs. The carcinoma in this vessel is also continuous with that shown in those photomicrographs. Carcinoma is present only in the lymph vessel of this newly formed tissue. Under the circumstances, just presented, it must be metastatic from the neoplasm in the preëxisting lymphatics of the tube. The condition, just shown, should not be confused with those pictured in Figs. 84, 85, 96, 102 and 120, where I believe that carcinoma implanted on the peritoneum gained access to the preëxisting lymphatics beneath it either by directly invading these vessels or else by first penetrating newly formed lymph vessels in the implants.  $\times 54$ .



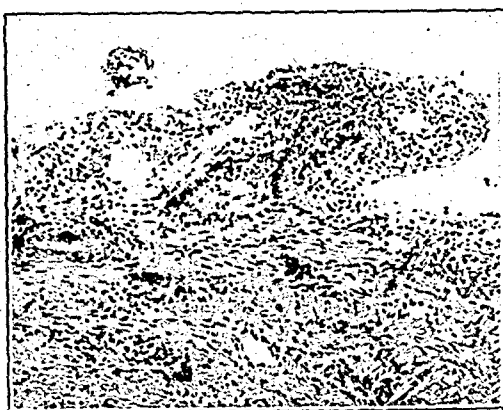
125



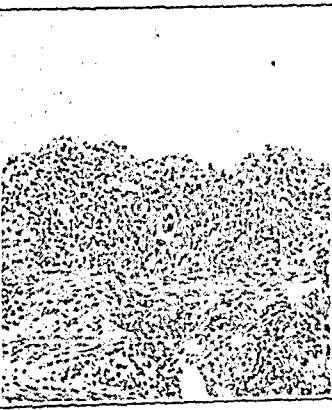
126



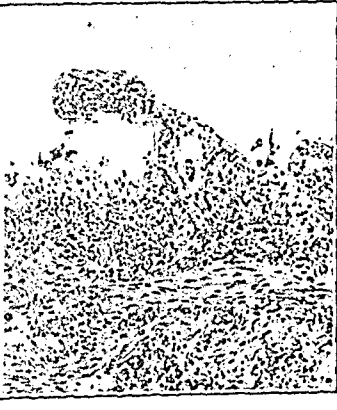
127



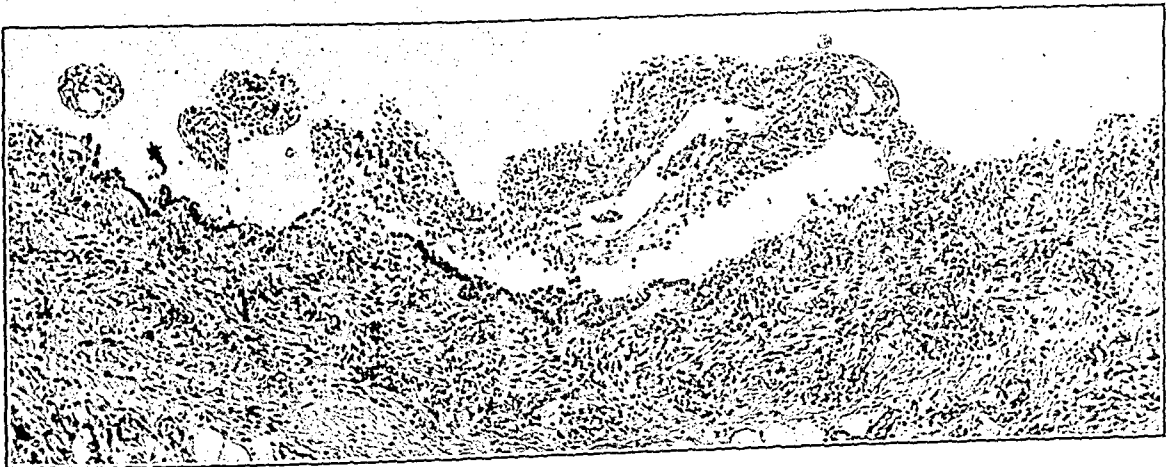
128



129



130



131





## LESIONS OF THE CARDIAC VALVE RINGS IN RHEUMATIC FEVER \*

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In a recent report by Gross and Kugel<sup>1</sup> on the topographical anatomy and histology of the valves in the human heart they described and defined precisely the topographical relations of certain areas which constitute the proximal portion of each valve leaflet and termed these the "valve rings." † In the above mentioned report the authors drew attention to the proximity of a pericardial wedge to most of the valve rings and indicated the strategic importance of the juxtaposition of these structures in the spread of infection. It was also pointed out that infection may spread by contiguity between the aortic, mitral and tricuspid valves, by way of the aortic annulus extensions (intervalvular fibrosa and septum fibrosum) which join these several structures. In support of the former, Friedberg and Gross<sup>2</sup> have recently shown that one of the mechanisms by which a rheumatic pericardial lesion may arise is by spread from a contiguous ring lesion.

In the few scattered reports dealing with lesions at the base of the valve no attempt was made to define accurately the ring as a topographical entity. Nevertheless, it is of interest to note that Holsti<sup>3</sup> found inflammatory processes in the valve root (Wurzel) and in the tissue surrounding the valve root in rheumatic hearts in which there was a deep valvulitis. Of greater importance is the report by Kugel and Epstein<sup>4</sup> in which these authors noted the presence of Aschoff bodies and diffuse inflammation in the musculo-arterial junction‡ of the pulmonic root in 17 of 24 cases of active rheumatic fever, and generally diffuse inflammation in the fibrous ring of the aorta (which corresponds to the musculo-arterial junction) in 22 of 24 active cases of rheumatic infection. In discussing the pathogenesis of these lesions Kugel and Epstein suggested that involvement of the pulmonary artery could come about (1) by direct extension along the

\* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.

† The term "ring" has been employed by other observers to indicate different sites, e. g. base of the valve leaflets, annulus, musculo-arterial junction, and so on.

‡ This area corresponds to the junction of the annulus with adjacent myocardium.

Received for publication February 5, 1936.

intimal wall, (2) by way of the vasa vasorum, and (3) by direct extension from inflamed pericardium. They stated further that "in the pathogenesis of valvulitis it is easily conceivable that the inflammation which has started in this vulnerable place (musculo-arterial junction) may extend up into the valve and there set up a corresponding reaction." In commenting on these observations Libman<sup>5</sup> suggested that the systolic murmurs heard along the left border of the sternum early in rheumatic heart disease may be due to valvulitis and, especially, disease of the mitral and possibly the pulmonic rings. The murmurs often disappear when a typical systolic murmur of mitral insufficiency develops (heard loudest at the apex and transmitted to the left).

Apart from the fact that no musculo-arterial junction exists in the auriculoventricular valves, considerable experience with histological studies of rheumatic fever material has convinced us that neither this site nor the roots of the valve show the earliest or most conspicuous lesions. In this and in a subsequent report<sup>6</sup> we propose to present evidence that indicates the valve ring, as defined by Gross and Kugel,<sup>1</sup> is the site of election for such lesions. It will be shown that in cases of acute rheumatic fever inflammatory lesions in the valve ring probably invariably precede the appearance of interstitial valvulitis. Indeed, the process may be limited to this area or may extend for a short distance only into the valve leaflet. For these reasons the valve ring must be considered a strategic site in the pathogenesis of rheumatic valvulitis, a focus from which other processes may extend.

This report will concern itself with a description of the gross and microscopic ring lesions that are found in active and in inactive cases of rheumatic fever, together with a discussion of the normal age period changes that this area undergoes in non-rheumatic hearts. Attention will also be directed to the intervalvular fibrosa,\* as well as to the subaortic and subpulmonic angles,\* since these latter areas are included under the definition of the valve ring. The changes

\* The intervalvular fibrosa (Fig. 1) represents the annulus extension of the aortic root which passes below the level of the posterior and left aortic cusps and is clothed by endocardium. It extends from the base of these aortic rings to the proximal termination of the anterior mitral leaflet. The subaortic and subpulmonic angles, collectively termed subvalvular angles, correspond to the endocardial tissue immediately overlying the angle made by the junction of the semilunar cusps with the subjacent structure (myocardium, annulus or septum fibrosum). For a detailed description of these structures see Gross, Antopol and Sacks.<sup>8</sup>

that take place in the remainder of the valve leaflet, in the valve pockets and in the chordae tendineae, will be taken up in a subsequent report.<sup>6</sup>

### MATERIAL AND METHODS

The material consisted of 40 non-rheumatic control hearts and 97 rheumatic hearts. Seventy-one of the latter were active and showed Aschoff bodies in the myocardium, and 26 showed chronic valvular disease of the typical rheumatic variety but without evidence of activity either clinically or pathologically, and with no demonstrable Aschoff bodies in the myocardium. The grouping as to activity and inactivity was based on the criteria outlined by Rothchild, Kugel and Gross.<sup>7</sup> Particular care was taken to avoid material that in any way indicated the possibility of a coexisting bacterial endocarditis. A careful study of the clinical records and pathological specimens made it possible to divide the rheumatic cases into the following groups:

GROUP I. Active cases where death took place during the first attack (12 cases).

GROUP II. Active cases where one preceding attack occurred within 1 year of the fatal outcome (7 cases).

GROUP III. Active cases where one previous attack occurred at least 2 years previous to the fatal outcome (11 cases).

GROUP IV. Active cases with a history of repeated attacks, death occurring during an acute recurrence (13 cases).

GROUP V. Active cases where death was caused by decompensation without clinical evidence of a final recurrence. In some of these cases there was no previous history of rheumatic fever (28 cases).

GROUP VI. Inactive cases of chronic valvular disease of the typical rheumatic variety (26 cases).

The sections from which these studies were made were cut according to the standardized technique of Gross, Antopol and Sacks,<sup>8</sup> and the technical procedures were those previously described by Gross and Ehrlich.<sup>9</sup>

TOPOGRAPHICAL RELATIONS OF THE SEMILUNAR VALVE  
RINGS \*

If one examines microscopically the semilunar cusps on cross-section (Fig. 1) it will be observed that the annulus fibrosis termination of the aorta and of the pulmonary artery splits into two limbs. One limb surrounds the sinus pocket and ascends the valve leaflet to form its fibrosa layer; the other is in close apposition to the adjacent myocardium and sends fibrous interdigitations into this structure. The line of junction between the myocardium and annulus forms the base of the semilunar cusp ring. It will be noted further that the two limbs of annulus, together with the endocardial covering of the valve leaflets at its base (ventricularis layer), generally enclose a triangular area occupied by a jelly-like tissue made up of transversely coursing collagen and elastic fibers with scattered stellate cells. This loose triangular area is the ring spongiosa. The cross-sectional limits of the ring may be defined as encompassing the roughly triangular area which includes the ring spongiosa with its ventricularis mantle, together with the two abutting limbs of annulus.

In the aortic rings the valve spongiosa is generally quite sharply defined; in the pulmonary rings, however, the separation of this layer from the fibrosa is usually not so sharp. Furthermore, elastic fibrils are not seen so often until later age periods, and the connective tissue feltwork is generally denser.

With regard to the existence of blood vessels in the semilunar cusp rings, our present observations confirm those of Gross and Kugel,<sup>1</sup> who found, in an examination of 100 normal hearts, that the aortic ring showed no vessels: in the pulmonic ring capillaries were observed in 7 per cent of the cases. These vessels are small and circular on cross-section, not surrounded by inflammatory cells, and in structure easily differentiated from granulation tissue capillaries. Furthermore, they almost invariably lie in the ring spongiosa.

The endocardium of the subvalvular angles (subaortic and subpulmonic) shows a slow progressive elastification. In the later age periods a small, oval, elastified reduplication may be found. This is sharply limited in its extent and never possesses blood vessels or inflammatory cells.

The intervalvular fibrosa consists of the downward extension of

\* For a more detailed description of these areas see Gross and Kugel.<sup>1</sup>

the aortic annulus which lies behind the posterior and left aortic cusps. It extends from the lower border of the aortic valve ring to the level of the auricular myocardial wedge tip and normally consists of dense collagen bundles with sparse cells and without blood vessels. The intervalvular fibrosa is covered by a more or less delicate layer of endocardium. In the later age periods the collagen becomes hyaline, and lipoid and calcific deposits may take place. Inflammatory cells and, in advanced lesions, granulation tissue may be seen in the neighborhood of these deposits.

With regard to the gross appearance of the semilunar rings, it may be stated that on cross-section these are extremely minute and sharply etched. Direct inspection of the ventricular aspect of the ring area shows a thin sharp line marking the insertion of the valve leaflet onto the adjacent myocardial structure. Not infrequently this sharp line shows a delicate depression which runs along the entire base of the valve leaflet. With advancing age the subvalvular angles develop a grayish appearance which is caused by a moderate elastification of the endocardium.

#### AGE PERIOD CHANGES IN THE HISTOLOGY OF THE SEMILUNAR VALVE RINGS

The large loose spongiosa of the aortic ring is frequently clearly defined by the third month of life. Elastification of the spongiosa generally becomes conspicuous from the fourth decade on, although it is occasionally seen considerably earlier. In the later age periods the structure becomes increasingly fibrillar, and toward the end of the sixth decade fat cells and dense collagenous bands not infrequently make their appearance. As a consequence the spongiosa of the aortic cusp may become rather small and dense, as well as heavily elastified. The annulus component of the ring shows no conspicuous changes until the later age periods when hyalinization, lipoid and calcific deposits, and even bone deposition may take place. The latter, as stated, is frequently surrounded by granulation tissue.

The pulmonic ring is, on the whole, considerably smaller than the aortic. In the earlier age periods it is frequently hardly discernible. However, from about the fifth year on it forms a somewhat fibrillar though still spongy zone. This develops elastification at about the same time as the corresponding aortic ring. Fibrillar changes con-

tinue to take place and, after the end of the fourth decade, the pulmonary ring is apt to be small, dense, collagenous and elastified.

### TOPOGRAPHICAL RELATIONS OF THE AURICULOVENTRICULAR VALVE RINGS

Since there is a wedge of myocardium (Fig. 1) inserted into the auriculoventricular cusps, a line drawn through the apex of this wedge at right angles to the auricular endocardium may be considered a simple means of defining the proximal limit of the auriculoventricular valve rings. In all the auriculoventricular cusps, with the exception of the septal flap of the tricuspid valve, cross sections of the ring may be arbitrarily considered as an inverted, triangular shaped portion of annulus in the immediate vicinity of the auricular myocardial wedge apex, limited above by the line referred to, which constitutes the base of this inverted triangle, and internally by the endocardium. It is found convenient to consider the triangle more or less equilateral and to take the width of the valve as a rough measure of each side. In contrast to all the other auriculoventricular valve cusps the septal flap of the tricuspid valve is inserted onto the annulus fibrosis or interventricular septum through the intermediary of a wide base. Here the ring may be conveniently considered as roughly rectangular in shape. The upper limit is the transverse line drawn through the apex of the auricular wedge; the lower limit is a similar line drawn at right angles to the endocardium at the level of the valve pocket; the outer limit is an imaginary line drawn parallel to the endocardium at a distance from it approximately equal to the width of the valve; and the inner limit is the endocardium.

With regard to the existence of blood vessels in the auriculoventricular rings, it may be stated that the anterior mitral valve ring possessed capillaries in 1 per cent of 100 cases examined by Gross and Kugel,<sup>1</sup> the posterior mitral ring in 2 per cent, and the septal leaflet of the tricuspid ring in 14 per cent. The incidence of capillarization of the remaining tricuspid leaflets is considerably lower. As in the semilunar rings, the mitral ring capillaries, when present, are extremely sparse and characteristically not of the granulation tissue variety. The vessels found in the septal leaflet of the tricuspid ring are generally more numerous, not infrequently take the appearance of somewhat dilated sinusoids, but still qualitatively show no evi-

dence of inflammatory origin. Inflammatory cells are normally not present in the auriculoventricular rings.

Gross inspection of the auricular aspect of the auriculoventricular rings discloses a clear-cut, delicate straight line which marks the insertion of the valve leaflet onto the adjacent myocardial structures. Although strands of auricular myocardium are occasionally seen extending into the base of the valve leaflets in the posterior mitral and tricuspid leaflets, particularly during the earlier age periods, the ring area is nevertheless quite clear-cut because of the rather abrupt termination of the bulk of the auricular myocardial wedge.

On cross-section the auriculoventricular rings (with the exception of the anterior mitral) show the same delicacy of structure as described for the semilunar rings. The anterior mitral ring is not clearly defined on gross inspection of the cross-sections and consists of the tissue immediately beyond the auricular myocardial wedge apex.

#### AGE PERIOD CHANGES IN THE HISTOLOGY OF THE AURICULOVENTRICULAR VALVE RINGS

The rings of the auriculoventricular leaflets consist, during the early age periods, practically entirely of annulus which becomes continuous with the fibrosa layer. The spongiosa is extremely inconspicuous. In the posterior mitral and tricuspid rings strands of auricular myocardium are not infrequently seen to dip fairly deeply toward the tip of the valve. This, however, is rarely seen after the first decade. Fat cells are occasionally found in the posterior mitral and in the tricuspid rings after the end of the fourth decade. In the later age periods the spongiosa layer is apt to show increased elastification and assumes a somewhat looser structure. The smooth muscle component of the auricularis layer of the auriculoventricular cusps also becomes more prominent with increasing age.

#### GROSS APPEARANCE OF RHEUMATIC VALVE RINGS

Definite gross abnormalities were present in the rings of one or more valves of the hearts of the first 5 groups. In the 6th group occasional cases appeared to have normal valve rings on gross examination. In general, the presence and severity of gross ring abnormalities corresponded to the extent of involvement of the

remainder of the valve — the mitral, aortic, tricuspid and pulmonic being affected in that order. In the majority of instances all four valve rings showed definite abnormalities.

The most frequent alterations were widening and irregularity of the superficial aspects of the rings. As a result the fine sharp boundary line between auricle and valve or between ventricle and valve became indefinite or hazy. Not infrequently the depression indicating the valve attachment was completely lost, the auricular or ventricular endocardium merging with that of the valve. As will be shown, these alterations in appearance are due to exudative and proliferative changes which fill out the ring boundary. Furthermore, inasmuch as considerable swelling, exudation, and later scarring, occur in the ring, the structure is not infrequently elevated above its previous level (*i.e.* toward the cardiac lumen) and, therefore, appears more prominent than normal. This is particularly true of the semilunar valves. Even in the presence of relatively little deformity of the semilunar cusps the normal translucency of the ring endocardium in the earlier age periods, and its delicate graying with age, were almost invariably replaced by a conspicuous "ground-glass" zone as the result of the formation of subvalvular reduplications. Occasionally there was gross vascularization of the ring region.

On cross-section the rings were frequently seen to be considerably thickened and sometimes swollen. In the cases of Group V and Group VI the rings frequently appeared normal on superficial examination but showed thickening on cross-section. In these same groups several of the cases revealed the presence of macroscopic lime in the ring of the mitral or aortic valve.

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVE RINGS IN GROUP I

##### *(12 Active Cases Where Death Took Place During the First Attack)*

In all 6 clinical groups of rheumatic fever investigated it was found that the most frequent site for the localization of the ring lesions was the ring spongiosa. In Group I the lesions were generally most extensive and consisted of a marked increase in capillaries and inflammatory cells (Fig. 2). Because of the short duration



of the ring lesions in this group, scarring, while present in a number of the cases, was not a conspicuous feature. The scarring manifested itself generally by an increase in the collagenous content of the spongiosa and a widening of the annulus as a whole.

As in the other groups, the lymphocyte was the predominating cell of the inflammatory exudate. Individual cases may show a predominance of polymorphonuclear leukocytes with a scattering of histiocytes, fibroblasts and, more rarely, mast cells. Infrequently the predominating cell was the young fibroblast. In approximately 10 per cent of each of the rings in this group, Aschoff bodies (Fig. 3) of the intercryptic mosaic variety were noted, particularly in the annulus portion of the ring. Occasionally eosinophilic swelling of the collagen (fibrinoid change) was also present.

In this group, vascularization by means of arteries and arterioles was extremely sparse. Furthermore, only one ring showed a vessel of the intimal musculo-elastic hyperplastic type.\* The paucity of muscular vessels indicates that the lesions were acute, that, as will be shown later, they were the result of a first attack of rheumatic fever, and that death took place after a short interval from the onset of the disease. Inasmuch as the average duration of the rheumatic bout in this series was approximately 6 weeks, it appears that more than 6 weeks are generally required for the development of muscular vessels in an inflammatory lesion resulting from rheumatic fever.

As will be shown later, in the absence of inflammatory cells it is at times difficult to determine whether the existing capillaries or vessels represent newly formed vascular channels or the residua of a scarred auricular myocardial wedge apex. This difficulty is encountered chiefly in the clinical groups in which activity of the lesion is at a minimum. However, even in the presence of scarring, isolated myocardial fibers usually determine the original extent of the wedge. The ring lesion should be considered as starting beyond the most distal of such isolated myocardial fibers.

In the auriculoventricular rings the spongiosa is generally limited in its extent. Particularly in the septal tricuspid ring, however, the spongiosa layer may be more conspicuous and this may be exaggerated by edema (Fig. 3). The lesion begins at the apex of the auricu-

\* For a detailed description of these vascular lesions occurring in rheumatic fever see Gross, Kugel and Epstein.<sup>10</sup>

lar myocardial wedge and involves the adjoining auricularis and fibrosa layers. In those instances in which the lesion was large it extended chiefly along the valve spongiosa zone and became continuous with a similar lesion within the valve leaflet (interstitial valvulitis). Furthermore, as frequently happened in this group, the lesion also diffusely involved the collagenous annulus which surrounds the valve pocket. Occasionally the newly formed capillaries localized around the tip of the auricular myocardial wedge.

In the semilunar valves, particularly the aortic, the capillaries were seen penetrating from the spongiosa into the surrounding annulus limbs. Not infrequently they approached the insertion of the great vessels and also extended down the intervalvular fibrosa. Of the 12 cases in this group, 7 showed capillarization and inflammatory cell involvement of the intervalvular fibrosa. As in the other groups to be described, the intervalvular fibrosa lesion consisted generally of capillaries irregularly distributed throughout the collagenous bundles with a tendency to localize close to the auricular myocardial wedge. These capillaries were surrounded by variable numbers of lymphocytes, occasionally by polymorphonuclear leukocytes and other mononuclear cells.

Subaortic lesions\* were found in 10 cases of this group. These consisted generally of a fibro-elastic layer overlying the dense elastic endocardial membrane and were histologically similar to what has been termed the elastified auricular endocardial reduplication (Gross<sup>11</sup>). These structures will be referred to in this report as subaortic reduplications. When they occur in the subpulmonic angle they will be termed subpulmonic reduplications. The mechanism of their formation is apparently similar to that described for the characteristic auricular reduplication. Indeed a number of the cases in this group showed active proliferative phenomena of the local fixed tissue cells, and stages in the transformation of these cells into connective tissue with subsequent elastification of the collagen. Various grades of inflammatory cell infiltration were associated with this process.

In a few cases multiple reduplications were noted (Fig. 4). These again bore a close resemblance to the multiple auricular endocardial reduplications. The oldest reduplications, *i.e.* those nearest the

\* Lesions were considered reduplications only when they exceeded in extent the elastification of the subvalvular angles, which occur in non-rheumatic hearts with increasing age, or when they showed qualitative differences from these.

original endocardium proper, were generally elastified. The most recent reduplications consisted of proliferated cells (mesenchymal (?)). The intermediate layer or layers were largely collagenous. The several layers were separated by dense elastic bands. In 4 cases the reduplications were vascularized. This process apparently consists of vascular penetration from the spongiosa and annulus portions of the rings into the subaortic reduplications. One case showed Aschoff bodies within the subaortic lesion, as well as a verrucous change on its endocardial surface.

Three of the cases in this group showed moderate subpulmonic elastified reduplications with rather sparse inflammatory cells. These reduplications were presumably of rheumatic origin. It will be noted that in all the groups to be discussed the subpulmonic lesions were generally milder than those occurring in the subaortic angle.

Of the 12 cases in this group, 10 showed involvement of all valve rings. In only 2 cases was one of the rings free of inflammatory changes. It must be noted, however, that these studies are based largely on one representative section from each ring. There is, therefore, a possibility that serial sections might have disclosed a lesion in every ring of each specimen. The two rings that presented no inflammatory lesion were the anterior mitral in 1 case, and the aortic in the other. The tricuspid and pulmonary ring lesions were in many cases the most severe.

## MICROSCOPIC APPEARANCE OF RHEUMATIC VALVE RINGS IN GROUP II

### *(7 Active Cases Where One Preceding Attack Occurred Within 1 Year of the Fatal Outcome)*

The ring lesions in this group were, on the whole, even more extensive than those in Group I. Moreover, they possessed certain distinctive qualitative differences from the latter (Fig. 5). A typical semilunar ring lesion showed considerable scarring of the spongiosa with increase in elastic fibers. Furthermore, the adjacent annulus limbs were so conspicuously widened by scar tissue that the ring as a whole showed macroscopic enlargement on cross-section. Between the meshes of this collagenous and elastic tissue there were frequently to be seen extraordinarily numerous inflammatory cells and

intensely engorged and dilated capillaries, thick walled arterioles, small arteries showing the typical intimal musculo-elastic hyperplastic changes, and occasionally considerable obliterating intimal fibrosis or channelling. The vessels, together with inflammatory cells, were dispersed throughout the surrounding annulus limbs and extended into the valve leaflets toward the roots of the great vessels and down the intervalvular fibrosa. The inflammatory cells were generally quite numerous and consisted chiefly of lymphocytes, but also of plasma cells, polymorphonuclear leukocytes and fibroblasts. The incidence of Aschoff bodies and eosinophilic swelling of collagen was approximately the same as in Group I.

Of additional interest is the fact that the intervalvular fibrosa showed inflammatory lesions in every case. Generally the involvement was fairly conspicuous. In 1 case (Fig. 6) it was so extensive and was associated with such pronounced whorling and hyalinization of the collagenous bundles that it suggested the presence of a syphilitic lesion (Sohval<sup>12</sup>).

The auriculoventricular ring lesions were qualitatively similar to those described for the semilunar rings. Here again the topographical differences in this site produced corresponding changes in the histological pattern. The rings were apt to be considerably scarred and showed elastification as well as elastica distortion. The annulus was usually definitely involved and, in the less active lesions, the capillaries showed compression and distortion. Frequently a conspicuous feature was the presence of widened, vascularized and inflamed auricularis reduplications in the ring region. These extended down the auricular surface of the auriculoventricular leaflets.

A characteristic feature of the Group II semilunar ring lesions was the frequency with which the vessels in the ring penetrated the elastic layer of the subvalvular angle and thus produced pronounced vascularization of the subvalvular reduplications to be described.

Six of the 7 cases in this group showed multiple, vascularized subaortic reduplications (Fig. 4). These consisted of superimposed zones of elastified and collagenous bundles separated by dense longitudinal bands of elastic tissue. Principally in the deeper layers of these multiple reduplications, numerous vessels, particularly of the intimal musculo-elastic hyperplastic type, were found. Generally the most superficial of these reduplicated layers showed the presence of smooth muscle bundles. As in Group I, inflammatory exudate

was, on the whole, sparse in the subvalvular angle. Four of the cases in this group showed subpulmonic lesions. These were less conspicuous than the aortic lesions and were multiple in only 2 cases. When the subvalvular reduplications were pronounced they were seen at times extending up the ventricularis aspect of the semilunar cusps, principally the aortic, to form a considerable thickening of the valve substance.

All the rings were involved in every case but 1 in this group. In this case only the aortic ring was free from any inflammatory process. As in Group I, the tricuspid and pulmonary ring lesions were frequently the most severe. The outstanding features of Group II ring lesions were the intensity of the inflammatory reaction, the frequent presence of intimal musculo-elastic hyperplastic vascular lesions, the multiplicity and vascularization of the subaortic reduplications, and the invariable involvement of the intervalvular fibrosa. A further aid in identifying ring lesions of this group was the frequent presence of intimal musculo-elastic hyperplastic vascular lesions in the adjacent myocardium.

### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVE RINGS IN GROUP III

#### *(11 Active Cases Where One Previous Attack Occurred at Least 2 Years Previous to the Fatal Outcome)*

In approximately half of the cases belonging to Group III the lesions were extensive and showed considerable activity. Thus, marked edema, Aschoff bodies and eosinophilic swelling of the annulus collagen were occasionally noted. In most of the rings the lymphocyte was the predominating cell. However, in 2 cases the exudate consisted largely of polymorphonuclear leukocytes, and in 2 other cases of plasma cells. In about half of the cases the lesions were considerably milder and were represented by scarring with distortion and compression of the residual capillaries. Intimal musculo-elastic hyperplastic vessels were rare. On the other hand, thickened and sometimes hyalinized arterioles were frequently noted. On the whole the rings were generally not as greatly widened as in Group II. Thus, the ring lesions in this group lacked the flagrant hypercapillarization seen in Group I and the frequent vascularization with intimal musculo-elastic hyperplastic lesions seen in Group II.

The subaortic angle was invariably involved in Group III. In 9 of the 11 cases this consisted of multiple elastified reduplications, in most instances vascularized. As in the ring lesion proper, intimal musculo-elastic hyperplastic lesions were infrequent. An Aschoff body was present in one subaortic lesion. In the subpulmonic angle lesions were found in approximately half the cases. Two of these consisted of multiple reduplications. In 1 there was an eosinophilic swelling of the collagen, and in only 1 was the lesion vascularized. Inflammatory changes in this area were considerably milder than in the subaortic angle. The intervalvular fibrosa was involved in all cases. The involvement was fairly extensive and in 2 cases, besides the capillaries, muscular vessels were noted.

Nine of the 11 cases showed involvement of each ring. In 1 of the 2 remaining cases the aortic ring was uninvolved and in the other the anterior mitral ring was uninvolved. On the whole, it appears that the pulmonic and tricuspid rings were somewhat more extensively involved than the other rings.

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVE RINGS IN GROUP IV

##### *(13 Active Cases Where Repeated Attacks Took Place, Death Occurring During an Acute Recurrence)*

The ring lesions in this group were similar to those in Group III (Fig. 7). There were, however, several additional features. Elastification and elastica distortion were often a more conspicuous feature. Intimal musculo-elastic hyperplastic vascular lesions were somewhat more frequent than in the previous group. The capillaries were often distorted by scarring, and hyalinized arterioles were frequently seen. In a number of cases the inflammatory cells tended to localize around the vessels.

The exudate in all cases consisted of lymphocytes. These were generally considerably sparser than those noted in the previously described groups. Aschoff bodies were seen in only two rings, one pulmonic and one tricuspid.

The subaortic angles were all involved and, with one exception, showed multiple reduplications. All the subaortic lesions showed vascularization. In 3 of the cases intimal musculo-elastic hyperplastic vessels were noted in the subaortic reduplications.

Only 4 cases showed subpulmonic reduplications. Two of these were of the multiple vascularized variety. Eleven of the 13 cases showed lesions of the intervalvular fibrosa. These were similar to those described in Group III.

Every case but 1 showed involvement of all the rings. In this case both the anterior mitral and the tricuspid rings showed no lesions. The tricuspid ring lesion was often quite severe.

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVE RINGS IN GROUP V

*(28 Active Cases Where Death Was Caused by Decompensation Without Clinical Evidence of a Final Recurrence. Some of These Cases Had No Previous History of Rheumatic Fever)*

In considering the lesions in this group it is to be noted that the clinical manifestations of rheumatic fever were, on the whole, much milder than in the previous groups. Furthermore, the age period in the average case was considerably older than that in the first 4 groups.

In this group the vascular lesions in the rings consisted of capillaries, thick-walled, often hyalinized arterioles, and small arteries with hypertrophied media or with intimal fibrosis. These lesions occurred with about equal frequency. In some cases all of these vascular lesions were present, in others only capillaries were noted. Not infrequently the number of vessels present was extremely few. Involvement of the surrounding annulus fibrosis was less frequent. Intimal musculo-elastic hyperplastic lesions were infrequent.

A conspicuous feature of the ring lesions in this group was the pronounced scarring and the dense and distorted elastification. The annulus was frequently hyalinized and showed a distinct paucity of cells. In the older age periods calcific deposition and bone formation were sometimes present in the ring.

Inflammatory cells were present in most of the ring lesions but were often exceedingly sparse. These consisted generally of scatterings of lymphocytes. In 2 cases the cells were fairly numerous and consisted of polymorphonuclear leukocytes. An Aschoff body was noted in only 1 case (pulmonic ring). In another case the posterior mitral ring showed eosinophilic swelling of collagen.

Subaortic lesions were present in only 12 cases. Almost invari-

ably these consisted of multiple vascularized elastified reduplications with a minimum of exudative phenomena. In a number of these cases the reduplications formed a thick mantle which became continuous with corresponding reduplications of the aortic valve ventricularis layer. Only 5 cases showed subpulmonic reduplications. In 1 of these an Aschoff body was present. Two of the lesions consisted of multiple elastified reduplications and in 2 cases the reduplications were vascularized. In the subaortic and subpulmonic angles, as well as in the mitral rings, large bundles of smooth muscle were often noted. The intervalvular fibrosa was mildly involved in 12 cases, the involvement consisting of sparse capillaries or occasional muscular vessels.

The incidence of ring lesions in this group showed a decided decline. Thus, whereas universal ring lesions were almost invariably noted in the first 4 groups, only 16 of the 28 cases in Group V showed involvement of all the rings. In 7 cases one ring only showed the absence of lesions. This occurred either in the aortic, anterior mitral, or pulmonic ring with about equal frequency. In the remaining 5 cases two or more rings showed no appreciable lesion. In no case in this group, however, was the tricuspid ring free of inflammatory involvement.

#### MICROSCOPIC APPEARANCE OF RHEUMATIC VALVE RINGS IN GROUP VI

##### *(26 Inactive Cases of Chronic Valvular Disease of the Typical Rheumatic Variety)*

The cases that fall into this group presented no appreciable clinical evidence of active rheumatic fever, in so far as this could be determined. Evidently, therefore, the course of the disease must have been extremely mild. A further point to be noted is that this group represents much older age periods than those previously described. As is to be expected, ring lesions in this group were less frequent than in the others, and when present were extremely indolent. Inflammatory cells were rare (Fig. 8). These consisted of lymphocytes, plasma cells or mast cells. Scarring and elastification were prominent. The vessels of the ring lesions consisted generally of sparse capillaries, hyalinized arterioles, or vessels with hypertrophied media. Intimal musculo-elastic hyperplastic vascular lesions



were not seen. In many cases only a few capillaries were present and the rings were totally devoid of inflammatory cells. The annulus collagen was frequently hyaline and contained lipoid crystals and calcific deposit. Not infrequently the most conspicuous lesion was present in the tricuspid or pulmonic ring.

The subaortic angle was involved in 15 of the cases. In 9 of these the lesions consisted of multiple elastified reduplications, and in 5 cases these were vascularized. Only one subpulmonic angle was involved. This showed a vascularized elastified reduplication. As in the rings, the subvalvular angles rarely contained inflammatory cells. Five of the 26 cases showed involvement of the intervalvular fibrosa. In 2 of these occasional arterioles were noted. In the remainder, capillaries and rare lymphocytes were present.

Of the 26 cases in this group only 6 showed involvement of all the rings (universal ring lesions). In 7 additional cases all the rings but one showed lesions. The rings that were most frequently devoid of lesions were the aortic and anterior mitral. The tricuspid ring was involved in every case but one. Incidentally this was the only tricuspid ring in the entire series of cases in the 6 groups that showed no evidence of inflammatory involvement, past or present.

## DISCUSSION

The occurrence of characteristic ring lesions in rheumatic fever hearts is of considerable interest and importance. Their almost invariable presence and acuity in the active stages of rheumatic fever, together with the fact that these rings may be the only part of the valve affected, suggest strongly that this is probably the first portion of the valve leaflet that is involved by the rheumatic process.

It seems, therefore, of interest to consider the mechanisms that may lead to the involvement of the valve rings. Since it has been shown that the normal mitral and aortic rings are practically devoid of vasculature and yet show an extraordinarily high incidence of ring involvement in rheumatic fever, it appears that an explanation which presupposes the presence of vasculature in the normal ring must be abandoned. There are left, therefore, four possibilities. One is the simultaneous contiguity spread from adjacent myocardium to all the rings. Another mechanism assumes initial involvement of one ring, most probably the mitral, this involvement taking

place by contiguity from the left auricular myocardial wedge. From this focus the infection may spread to other rings by way of the adjacent pericardial wedge, as well as along the annulus extensions of the intervalvular fibrosa and septum fibrosum which form a close intercommunication between the mitral, aortic and tricuspid valve rings. Although the pulmonic ring is not joined to the others through the annulus extensions common to them, it is in close apposition to the aortic ring through a narrow pericardial bridge which links the left-right commissures of both of these valves. Contiguity infection of the pulmonic ring is thus facilitated. A third possible mechanism is the involvement of the aortic and pulmonic rings by contiguity spread from the aortic and pulmonic root lesions which have been shown (Gross<sup>13</sup>) to occur frequently, particularly during the active stages of rheumatic fever. From these foci spread may take place as indicated above. A fourth possibility is the simultaneous occurrence of the three above mentioned mechanisms.

The most satisfactory solution of this problem requires the experimental reproduction of this disease. This, however, has not been accomplished as yet. Another approach to the problem would be to study the ring lesions in cases where death occurred during a first attack of rheumatic fever, within a few days after the onset of the disease, in order to follow the sequence of events. The shortest attack that we were able to study in our series lasted 2 weeks. It must be noted, however, that the onset of the disease can rarely be definitely fixed. Furthermore, it is difficult to determine with certainty whether or not a previous attack had taken place. Consequently, the fact that almost invariably all the rings showed lesions in our earliest first attack cases does not necessarily indicate simultaneous involvement of all the rings, but may represent a very rapid spread from one ring, possibly the mitral, to the others along the pathways mentioned above.

In support of the view that the initial lesion may occur in the mitral ring by contiguity from the left auricular myocardial wedge are: (1) the almost invariable and severe involvement of the left auricle in rheumatic fever, particularly during the active stages (Gross<sup>11</sup>); (2) the considerably less frequent and less intense involvement of the right auricle; (3) the fact that the left auricular myocardial wedge apex shows more marked inflammatory changes and vascular involvement than any other part of the left auricle;

and (4) the fact that in the rather infrequent cases in which rheumatic lesions are strictly confined to one valve the mitral is the one usually involved. These findings, however, are not necessarily inconsistent with simultaneous involvement of all the rings, providing it is assumed that once infection has taken place in the left and right auricles, other factors (*e.g.* the somewhat greater tension within the left auricle than in the right) may permit of more extensive spread of the disease in the former. In support of the contention that the lesions spread by contiguity through the annulus extensions are the frequency with which inflammatory changes are found in the intervalvular fibrosa and septum fibrosum (Gross and Fried<sup>14</sup>) in active rheumatic fever. Coombs<sup>15</sup> has already observed that Aschoff bodies "are particularly fond of the tracts of muscle which border on the central fibrous body."

On the other hand, in recent studies by Gross and Friedberg<sup>6</sup> and by Sohval and Gross,<sup>16</sup> it was found that the intervalvular fibrosa was seldom involved in Group VI cases (inactive or healed rheumatic) in spite of the fact that the mitral, tricuspid and aortic valves or rings almost invariably showed evidences of a healed rheumatic process. This indicates either that simultaneous involvement occurred in all the rings but, because of the indolence of the inflammatory process in this group, complete healing took place in many; or that an initial mitral ring infection was spread by contiguity through the annulus extensions with subsequent complete healing in the latter and restitution to integrity in most of the cases. The first of these explanations seems the more likely.

That healing undoubtedly plays a rôle in limiting the spread of a rheumatic infection is well exemplified in the tricuspid and pulmonic valves. It has been shown that lesions occur in these sites with considerable frequency and are often even more extensive than ring lesions within the left heart. Assuming that ring lesions precede interstitial valvulitis, the lower grade and lesser incidence of lesions of the valve leaflets in the right heart indicate that other mechanisms must come into play. It may be, as suggested above, that the lower tension in the right heart or, perhaps, the venous blood which bathes the valves, constitutes a less favorable environment for the progress of the rheumatic infection. Of these two, tension undoubtedly plays the major rôle. This is strikingly illustrated in the rather rare cases of indolent rheumatic infection (Group VI) asso-

ciated with hypertension of the lesser circulation as the result of pulmonary disease (emphysema, and so on). In such cases the tricuspid valve may show marked deformity, although the mitral and aortic valves may be relatively intact grossly and present only microscopic evidences of disease.

Apart from these considerations on the mechanism concerned in the development and spread of a rheumatic ring lesion, its occurrence is of considerable significance in the diagnosis of rheumatic involvement of the heart. This is true in spite of the fact that ring lesions occur in other conditions, such as atypical verrucous endocarditis of Libman and Sacks<sup>17</sup> (Gross<sup>18</sup>), luetic aortitis with involvement of the aortic commissures (Sohval<sup>12</sup>), and subacute bacterial endocarditis. The rheumatic ring lesions, however, generally possess certain qualitative and quantitative characteristics which, with other associated findings, distinguish them from those occurring in the above mentioned conditions, providing a coexisting rheumatic process can be ruled out. Thus, whereas the rheumatic ring lesions present no single specific component (except Aschoff bodies when they are present) the structure as a composite picture is frequently such as to leave no room for doubt concerning its rheumatic nature.

Although there are no clear-cut differences that distinguish sharply the ring lesions in the several groups of rheumatic fever described, each group presents as a whole more or less characteristic features. This is in keeping with the findings previously reported by Gross and collaborators in other cardiac sites. Briefly considered, the characteristics of the ring lesions in each group were as follows:

In Group I the lesions were most extensive, consisting of marked capillarization and infiltration with inflammatory cells, sometimes with edema. Blood vessels of the muscular or intimal musculo-elastic hyperplastic type were infrequent. Aschoff bodies were present in about 10 per cent of the rings. There was little scarring. Practically all the rings and subaortic angles showed lesions. In the latter site, reduplications, when present, were generally not multiple. Approximately half the cases showed involvement of the inter-valvular fibrosa.

In Group II the lesions were also extensive and consisted of considerable infiltration and vascularization. The latter frequently consisted of intimal musculo-elastic hyperplastic lesions. The inflammation spread in all directions. The incidence of Aschoff bodies

was approximately the same as in Group I. Scarring was pronounced. Practically all rings showed involvement. The highly characteristic, multiple, subaortic vascularized reduplications occurred in practically every case. The intervalvular fibrosa was invariably involved.

The lesions in Group III were somewhat milder. The vascular lesions consisted of capillaries and muscular vessels in about equal proportions. In some cases vascularization was by means of capillaries only. The incidence of Aschoff bodies was lower than in the previous groups. Intimal musculo-elastic hyperplastic lesions were infrequent. Practically all rings showed involvement. The subaortic angle invariably showed reduplications. In most instances these were multiple vascularized. This group showed the highest incidence of subpulmonic lesions, *i.e.* in approximately half the cases. The intervalvular fibrosa was invariably involved.

In Group IV the lesions were somewhat similar to those noted in Group III. Intimal musculo-elastic hyperplastic lesions were somewhat more frequent than in Group III. Not infrequently the ring lesions consisted only of distorted capillaries as the result of the scarring process. The occurrence of inflammatory cells was less evident than in the previous groups. Aschoff bodies were infrequent. All the rings were involved. All the subaortic angles showed lesions that were almost invariably of the multiple elastified variety. These, however, were not as pronounced as in the first two groups. The intervalvular fibrosa showed a high incidence of lesions (11 or 13 cases).

Group V showed a considerable diminution in the extent, intensity and incidence of the lesions. The blood vessels were generally thick walled arterioles or arteries, or distorted capillaries. The rings showed advanced scarring and elastica distortion. Cellularity was sparse and Aschoff bodies rare. Subaortic lesions occurred in approximately half the cases. These were generally of the multiple vascularized variety. The incidence of the intervalvular fibrosa lesion was approximately 50 per cent. These were generally mild. In contrast to the previous 4 groups, universal ring lesions occurred in approximately half the cases. Ring lesions were found in three rings in another 25 per cent of the cases. Every case showed involvement of at least two rings.

In Group VI exudative phenomena were practically nil. Aschoff

bodies were not present. Inflammatory cells were extraordinarily sparse, scarring was pronounced, and vascularization consisted of capillaries, hyaline arterioles or hypertrophied vessels. Subaortic lesions occurred in approximately half the cases. Half of these were multiple elastified reduplications, and only half of these again were vascularized. Only 1 case of the 26 in this group showed a subpulmonic lesion. Intervalvular fibrosa lesions were found in only 5 of the cases. These were extremely mild. Only 6 cases showed universal ring involvement and in another 7 three rings were involved. At least one ring was involved in every case.

Considered as a whole, it is seen that the most active and vigorous inflammatory lesions occur in Groups I and II. Each of these groups possesses definite characteristic features. Groups III and IV show a diminution in inflammatory phenomena and, therefore, occupy an intermediate position in these clinical subdivisions. In Groups V and VI there is an abrupt diminution in the extent of inflammatory lesions, particularly of the exudative phenomena. Furthermore, whereas lesions are present in almost every ring in the first 4 groups (universal ring lesions), they show a definitely diminished incidence in Group V and are not infrequently absent in many of the rings in the Group VI cases.

The frequent occurrence of subvalvular angle lesions of the semi-lunar cusps, particularly the aortic, merits repetition. In the more chronic groups the presence of characteristic multiple, often vascularized elastified reduplications is of considerable value in making a diagnosis of rheumatic lesions and in estimating the extent of the damage wrought.

A practical application of these findings lies in attempting to trace the etiology of obscure lesions such, for example, as the so-called Mönckeberg's ascending sclerosis of the aortic valve.\* Having shown that even in Group VI the presence of ancient rheumatic fever shows involvement of at least four of the five rings studied (the mitral was represented by two sections), and of the subaortic angles in half the cases, one would expect that, if rheumatic fever is the underlying basis of Mönckeberg's ascending sclerosis of the aortic valve, these associated phenomena of an extinct rheumatic lesion would be present in a representative number of cases of that disease. When one adds to this the fact that in a large proportion of rheumatic

\* A report on this problem is in the course of preparation (Sohval and Gross<sup>16</sup>).

hearts one may expect to find other evidences of previous rheumatic damage, such as myocardial,<sup>9,19</sup> vascular,<sup>10</sup> root,<sup>13</sup> auricular,<sup>11</sup> conduction system,<sup>14</sup> pericardial<sup>2</sup> and valve<sup>6</sup> lesions (Gross *et al*), one has a considerable armamentarium with which to attempt to arrive at a reasonable solution as to whether or not rheumatic fever is the underlying basis of the pathological lesions studied.

Finally, inasmuch as the ring constitutes the proximal portion of the valve leaflet, the evidence presented in this report proves conclusively that involvement of only one or two valves in rheumatic fever is by far the exception. On the contrary, in the great majority of cases all the valves are involved. The involvement, however, apparently is qualitatively of such a nature that while it is progressive chiefly in the mitral and aortic cusps, its progress is markedly diminished in the tricuspid and, more particularly, in the pulmonic valve. The result of this is that whereas the incidence of microscopic valvular lesions in all four valves is surprisingly high, the extent of involvement of these lesions in the right heart is frequently such as to lead to no conspicuous macroscopic changes.

Apart from the microscopic findings, the descriptions that have been presented of the gross features of the rings and subvalvular angles should constitute a useful addition to our knowledge of the gross changes occurring in the human heart valves in rheumatic fever.

#### SUMMARY AND CONCLUSIONS

There have been described in this report the incidence, and gross and microscopic appearances of lesions in the valve rings and inter-valvular fibrosa occurring in 97 cases of rheumatic fever. These cases have been divided into 6 clinical groups which represent various courses taken by this disease. It has been shown that each group presents certain general gross and microscopic features that bear a relation to the clinical grouping. New macroscopic and microscopic data are presented on the development of the rheumatic lesions in the valve rings, and their significance with regard to the spread of the rheumatic infection to and from these sites is discussed. It is shown that the findings herein presented, as well as those previously reported, are of value in elucidating the pathogenesis of other cardiac lesions. Inasmuch as the valve ring constitutes the proximal portion of the valve leaflet, the ring lesions are

of considerable significance in studying the development of rheumatic valvulitis. A description is also given of the changes that take place in non-rheumatic valve rings during the first eight decades of life.

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## DESCRIPTION OF PLATES

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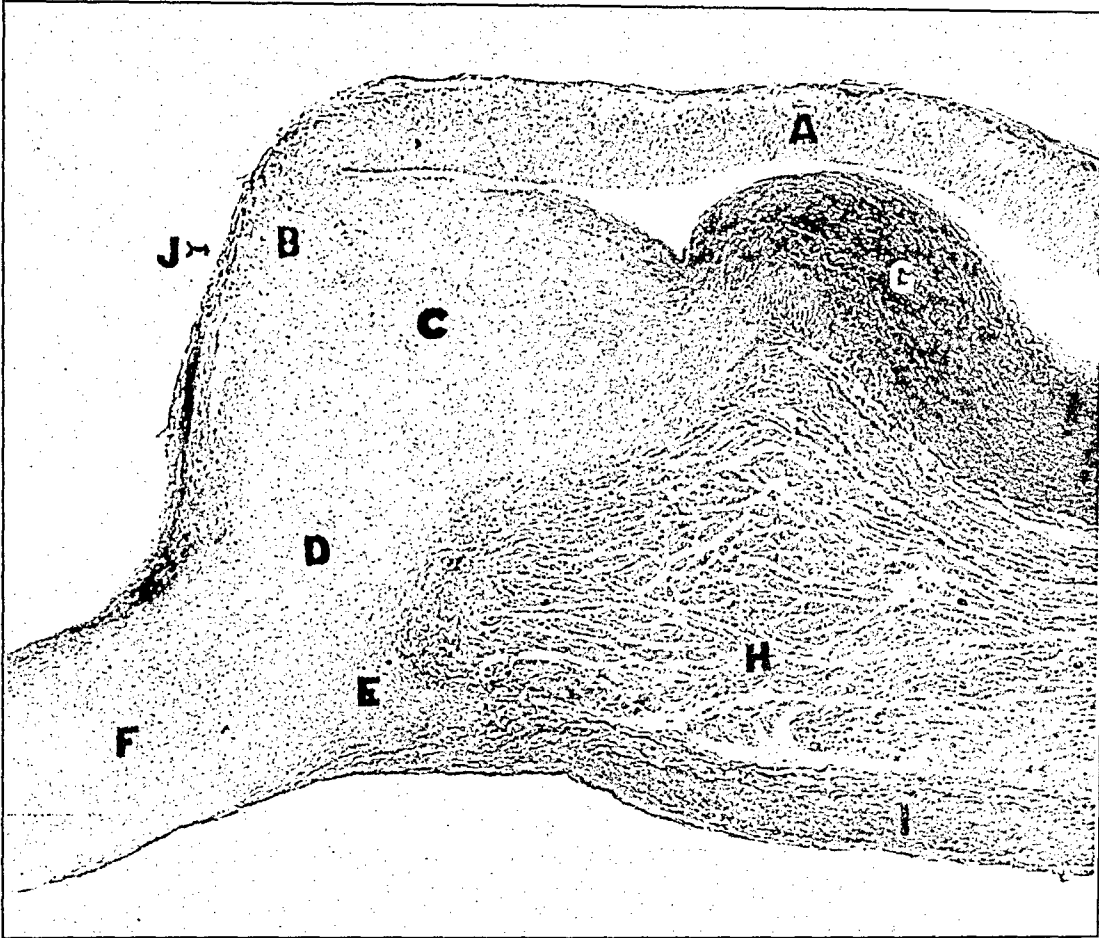
### PLATE 91

FIG. 1. Cross-section of normal posterior aortic and anterior mitral rings. Age 18 months. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

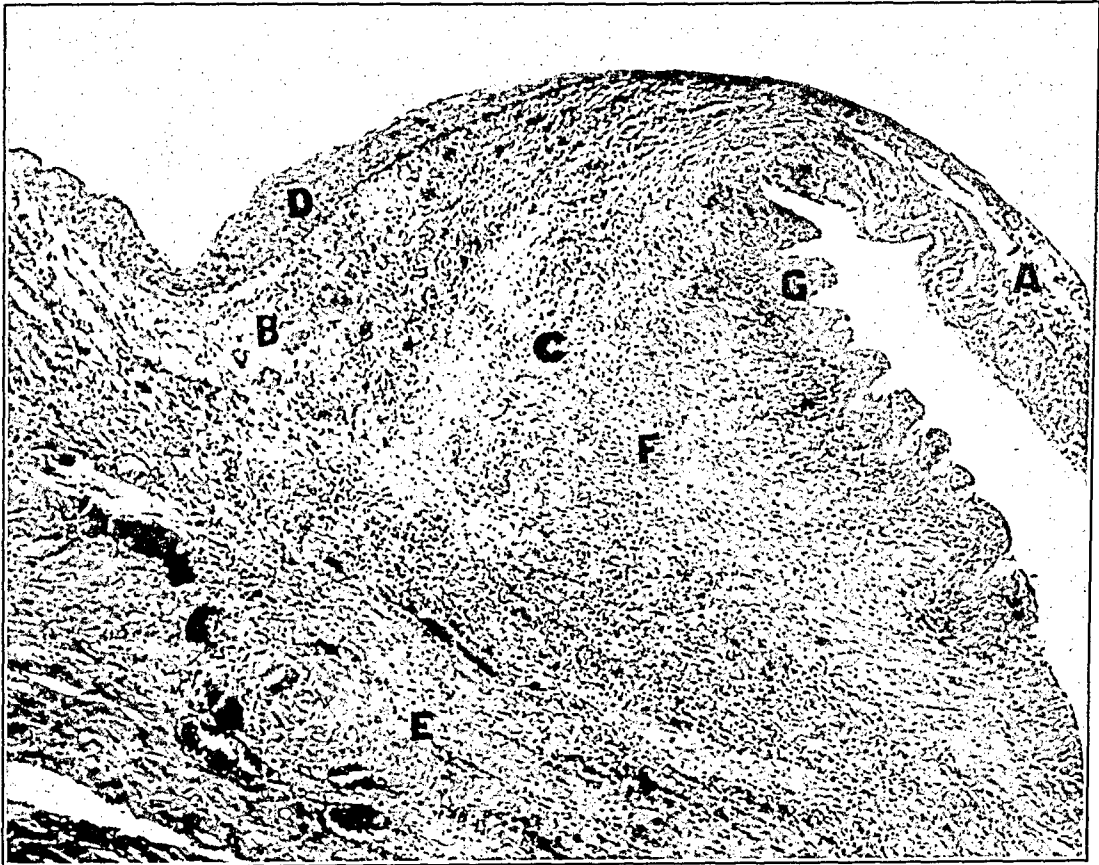
A = posterior aortic leaflet; B = aortic ring spongiosa; C = aortic ring annulus; D = intervalvular fibrosa; E = anterior mitral ring; F = base of anterior mitral leaflet; G = root of aorta; H = left auricular myocardial wedge; I = left auricular endocardium; J = subaortic angle covered by the ventricularis layer.

FIG. 2. Cross-section of pulmonic ring in a case of active rheumatic fever (Group I). Age 5 years. Low power. Hematoxylin and eosin stain.

A = pulmonic leaflet; B = ring spongiosa showing edema, capillarization and infiltration with inflammatory cells; C = pulmonic ring annulus showing marked inflammation and capillarization; D = moderate sub-pulmonic reduplication; E = inflamed myocardium with injected vessels; F = eosinophilic swelling of annulus collagen; G = eosinophilic swelling of elastic lamella in pulmonic pocket. Note early formation of pocket polypi.



I



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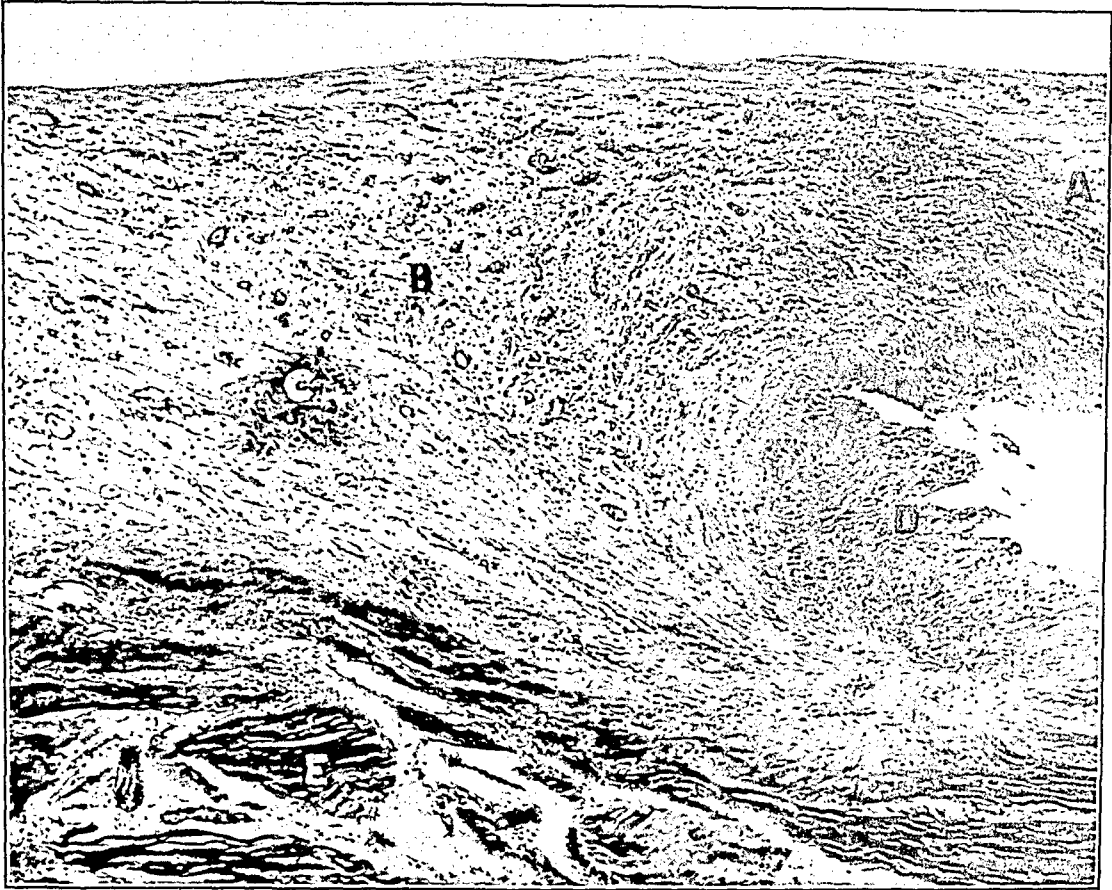
PLATE 92

FIG. 3. Cross-section of tricuspid ring in a case of active rheumatic fever (Group I). Age 10 years. Low power. Hematoxylin and eosin stain.

A = base of tricuspid leaflet; B = tricuspid ring showing marked edema, hypercapillarization and infiltration with inflammatory cells; C = eosinophilic swelling and fusion of collagen with early Aschoff body formation; D = tricuspid pocket showing polypoid formation; E = myocardium with interstitial inflammation.

FIG. 4. Cross-section of aortic ring in a case of active rheumatic fever (Group II). Age 14 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic leaflet; B = scarred ring containing numerous vessels. Many of these are of the intimal musculo-elastic hyperplastic type. C = aortic ring annulus containing blood vessels; D = aortic ring annulus near junction of myocardium. Note marked vascularization and intimal musculo-elastic hyperplastic vessels. E = myocardium; F = multiple vascularized elastified subaortic reduplications.



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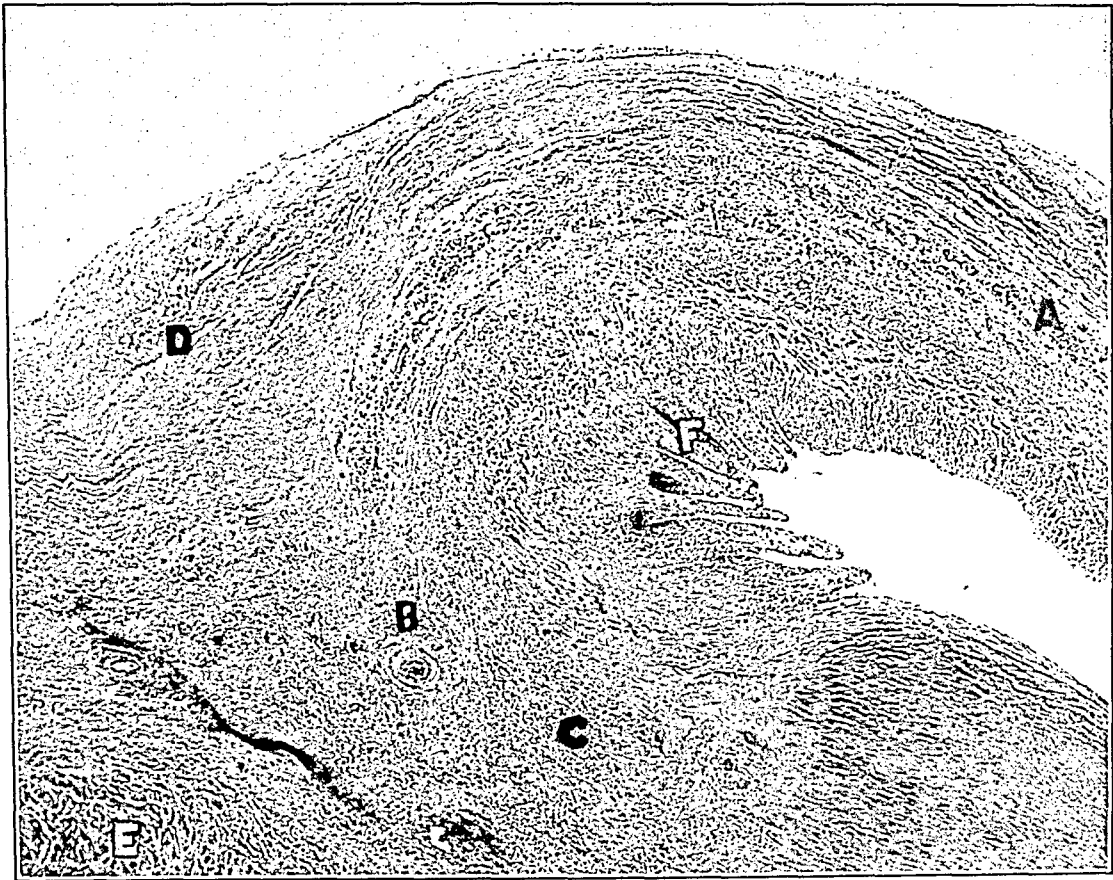
PLATE 93

FIG. 5. Cross-section of aortic ring in a case of active rheumatic fever (Group II). Age 18 years. Low power. Hematoxylin and eosin stain.

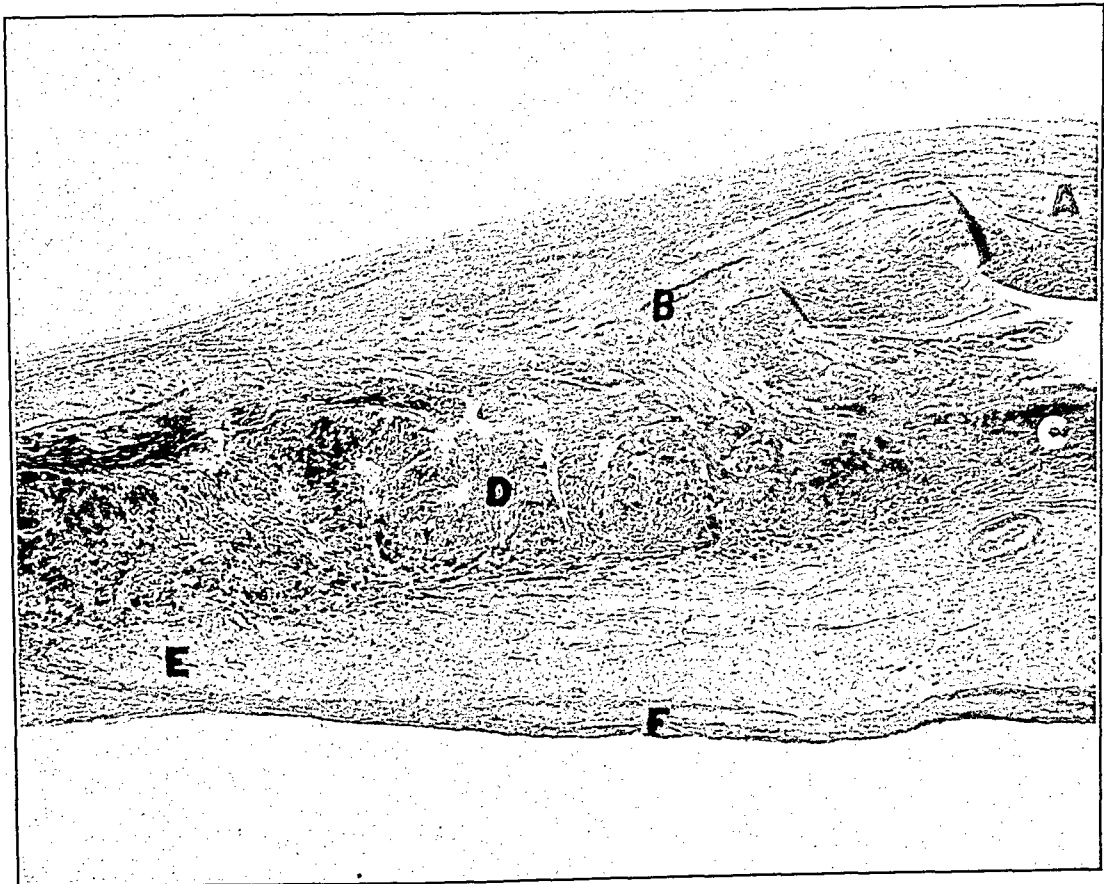
A = aortic leaflet; B = scarred ring spongiosa showing increased inflammation and vascularization. Note intimal musculo-elastic hyperplastic vessel. C = aortic ring annulus with inflammation; D = subaortic reduplications; E = myocardium; F = polypoid formation in aortic pocket.

FIG. 6. Cross-section of posterior aortic and anterior mitral leaflets showing intervalvular fibrosa in a case of active rheumatic fever (Group III). Age 17 years. Low power. Masson's erythrosine-saffron stain.

A = posterior aortic leaflet; B = markedly scarred aortic ring; C = root of aorta; D = intervalvular fibrosa. Note pronounced whorling of collagen, vascularization and scar formation. E = tip of left auricular myocardial wedge; F = left auricular endocardium.



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PLATE 94

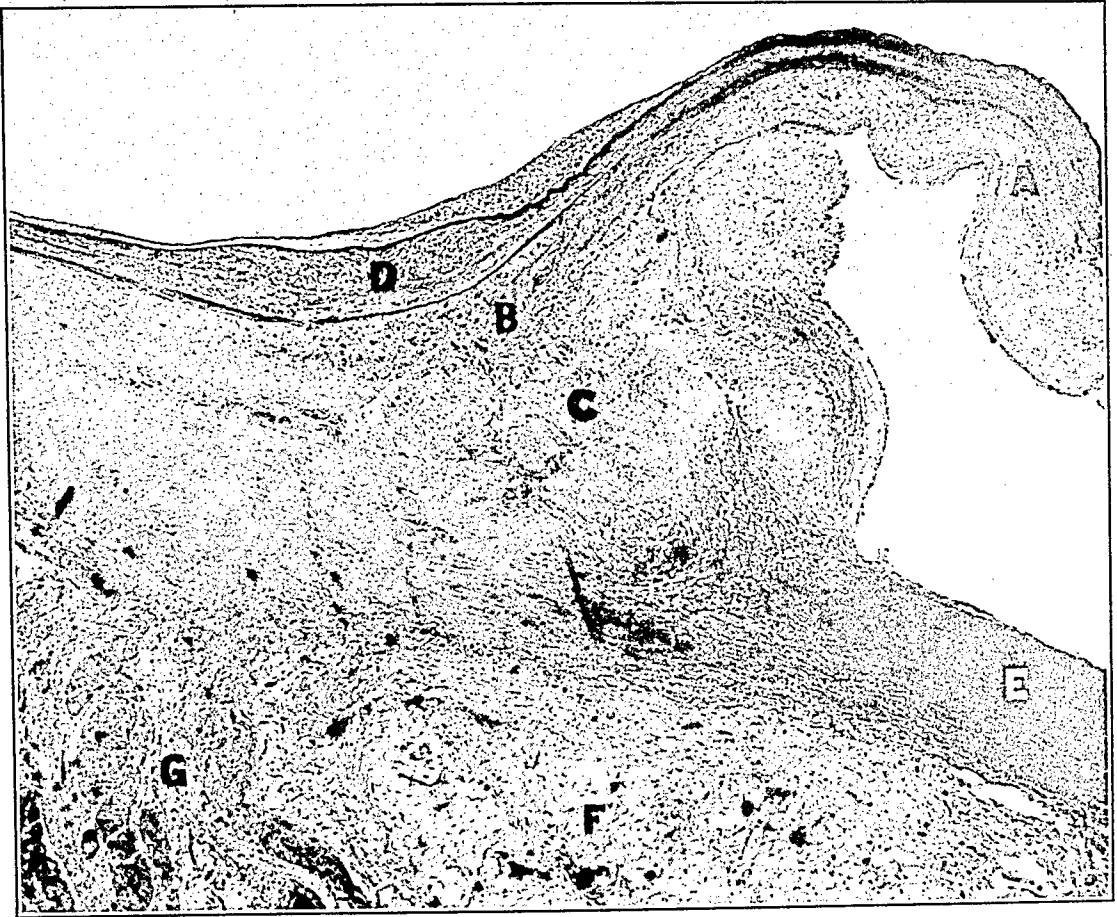
FIG. 7. Cross-section of aortic ring in a case of active rheumatic fever (Group IV). Age 14 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic leaflet; B = scarred aortic ring spongiosa. Note distorted capillaries and paucity of cells. C = aortic ring annulus containing distorted capillaries and arterioles; D = multiple subaortic elastified reduplications; E = root of aorta; F = retro-aortic pericardial wedge showing scarring; G = scarred myocardium.

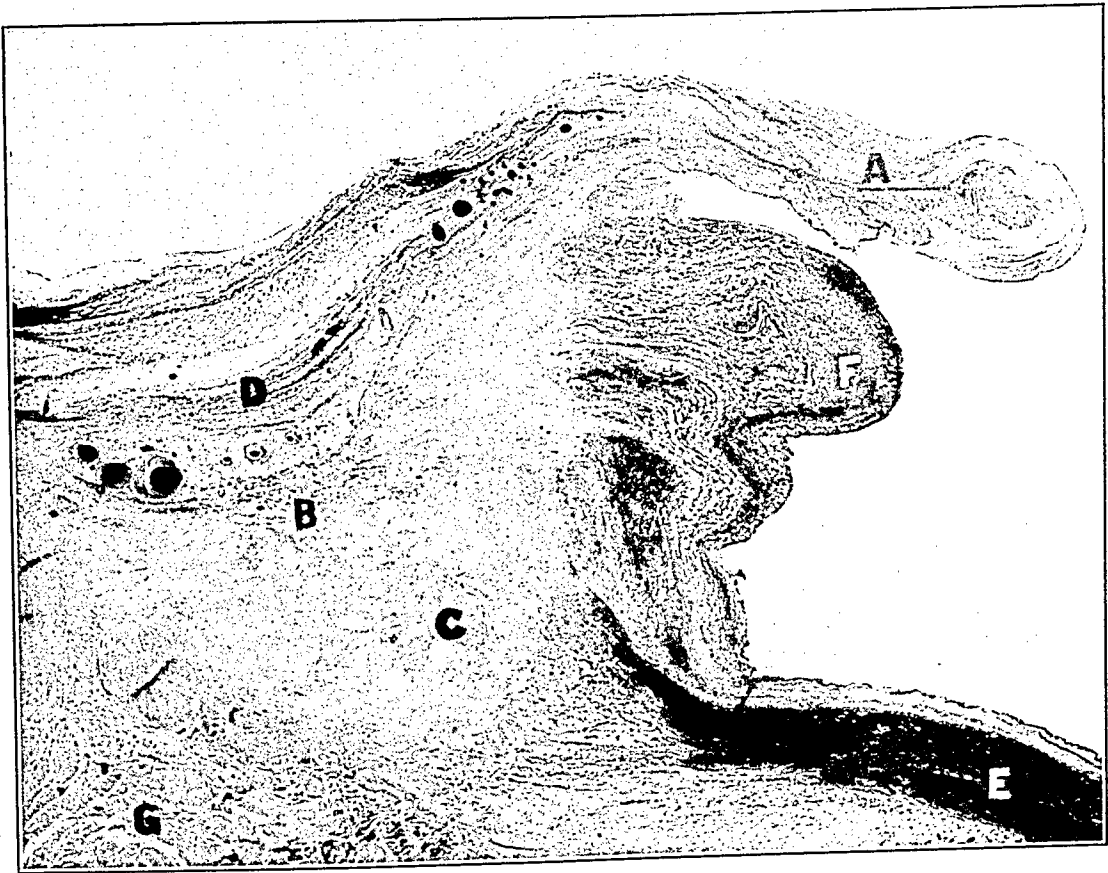
FIG. 8. Cross-section of aortic ring in a case of inactive rheumatic fever (Group VI). Age 21 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic leaflet; B = completely scarred ring spongiosa showing few capillaries; C = aortic ring annulus with rare capillaries; D = multiple elastified vascularized subaortic reduplications. Note thick-walled vessels. E = root of aorta; F = elastified pocket reduplications; G = myocardium.





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# THE HISTOPATHOLOGY OF EXPERIMENTAL MUMPS IN THE MONKEY, *MACACUS RHESUS* \*

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The pathology of epidemic parotitis in the human is still indefinitely known, though the disease was recognized and a classical clinical description was given by Hippocrates. In part, this has been due to the fact that death from epidemic parotitis is extremely rare. Also, examinations of the gland by biopsy during the disease have been quite limited. For these reasons the opportunity to observe the changes in the gland itself during the disease has been limited to very few and isolated studies.

As we have not had the rare opportunity to examine the parotid glands of humans during the course of this infection, either by biopsy or postmortem examination, we cannot add directly to the present conceptions of the pathological picture of the disease in the human. However, by the presentation of the pathological picture of the experimental disease in *Macacus rhesus* monkeys, proved to be due to the virus of epidemic parotitis, and by comparison with the reported studies of the disease in humans, we hope to add further knowledge to the pathology and pathogenesis of the lesions in the glands.<sup>1,2</sup> There will be no attempt to summarize the literature completely, but the observations and views of the more recent authors will be given in an attempt to present the present concept of the changes in the parotid gland in this infection.

Dopter and Repaci in 1909 reported a partial postmortem examination on a soldier who had died on the 6th day after coming under observation for acute parotitis.<sup>3</sup> He had developed an acute orchitis on the 4th day, at which time the parotid phenomena had been found to be slightly diminished. The parotids were enlarged, of a rosy color and slightly congested, and on sectioning a few hemorrhagic spots were seen. Microscopically the fibers of the thickened interstitial connective tissue, greatly infiltrated by leukocytes, were denser than normal. The leukocytic infiltration was most abundant

\* Aided by grants from the Josiah Macy Jr. Foundation, and the Division of Medical Sciences, Rockefeller Foundation.

Received for publication December 14, 1935.

about the blood vessels. The glandular lobules were irregularly infiltrated by white blood cells. The glandular epithelium, though swollen and edematous, did not appear to be the seat of notable degenerative alterations. The excretory ducts were unequally altered; the smaller ducts being little or not at all changed, while the larger collecting ducts showed considerable changes. These changes consisted in infiltration of the walls by leukocytes, desquamation of both healthy and necrotic epithelial cells, and the presence of an albuminous substance and in some instances polymorphonuclear cells within the lumens.

Cornil and Ranyier in 1912, in a section which had been given them, observed an edematous exudate infiltrated between the acini in the neighborhood of the excretory canals.<sup>4</sup> The acini in these areas were seen to be diminished in volume on account of cellular compression. However, the predominant lesions were thought to be those of the excretory ducts. The cells of the walls were swollen, fusiform, and dissociated in part by edema. Some were detached and mixed in the lumen with leukocytes in part of the polymorphonuclear type. At short distances from these ducts the acini appeared unaltered. An intense, splotchy vascular congestion between the acini also is described as being present.

Delater<sup>5</sup> and Reverchon, Worms and Delater<sup>6</sup> in 1922 reported the examination of the submaxillary and sublingual glands in a case of sudden death from laryngeal edema, which appeared 8 days after the patient had been dismissed from the hospital following a classical evolution of epidemic parotitis. The tissues surrounding the glands were edematous and the glands themselves were seen to be notably altered. The capsule was found to be only slightly infiltrated. The interstitial connective tissue was somewhat edematous, so much so that numerous spaces were present. Along the vessels and ducts where the stroma was denser there were foci of large mononuclear infiltration which in some areas were rather large and poorly outlined. The acini were uniformly stained and without apparent lumens. The acinar cells were swollen, and possessed indistinct outlines which caused the acini to appear as grayish violet staining, granular cytoplasmic masses in which there were scattered poorly stained nuclei. Some of the cells were separated from the basal membrane, and in places only skeletons of previous acini remained. Exceptionally, leukocytes could be found in the acini. The excre-

tory canals were altered also; the cells were detached from the basal membrane and lay free in the lumen, and leukocytes had invaded the walls, but were not present in the lumen.

The process is summarized by the authors as being a profound and massive alteration of the epithelium of the acini and of the excretory canals, a leukocytic infiltration in which no polymorphonuclears took part, and an inflammatory edema which exceeded the limits of the glands.

Rocchi in 1933 gave a summary of the literature along with his own observations on the pathology of mumps with special reference to the salivary and lacrimal glands, pancreas and testicle.<sup>7</sup> His observations on the salivary glands were made on biopsies of the glands from 4 cases. The material consisted of pieces of the parotid from 3 cases, 2 of which were in the 2nd day, and 1 in the 4th day of illness at the time of biopsy. The material from the 4th case was obtained from a submaxillary gland on the 4th day of illness.

The excised fragments were found to be of a grayish red color with scattered, small hemorrhagic areas. Microscopically the alterations in the gland were variably dispersed. The alterations were either well localized in circumscribed areas or diffused more extensively. Although the prevalent lesions seemed to be distinctively interstitial and peritubal, there were also alterations of the parenchymal cells. These alterations consisted in condensation with a resulting deeper staining chromatin of the nucleus and a bluish diffusion of hematoxylin within the cytoplasm of the mucous cells, which may be interpreted as evidences of early necrosis. The excretory ducts in 2 of the parotid cases showed distinctly degenerative changes which consisted of the formation of vacuoles in the cytoplasm and displacement, pyknosis or complete disappearance of the nuclei. There were polymorphonuclears, a few red blood cells, desquamated epithelium and cellular fragments. The other 2 cases showed no such degenerative changes and only a granular eosinophilic staining material was found in the lumens of the ducts. The interstitial inflammatory process and the tubular changes seemed independent of each other. The interstitial alterations consisted of a serous exudate, a marked infiltration by large mononuclear and lymphocytic cells, and the hemorrhagic areas that had been noted in the gross examination of the fragments. The lymphocytic infiltration was present in both interacinous and peritubular inter-

stitial tissue. Often there were accumulations of these cells having the appearance of solitary lymph follicles, while in other areas they were more diffusely distributed. Vascular congestion was also noted.

From a review of the literature we may construct the following composite picture of the changes described as occurring in the process of involvement of the parotid gland by the virus of epidemic parotitis: an interstitial serous exudate (Niemeyer<sup>8</sup>); an interstitial fibrinous exudate (Bamberger<sup>9</sup>); catarrhal inflammation of the excretory ducts (Virchow<sup>10</sup>); foci of hemorrhage, an interstitial infiltration by leukocytes, desquamation of the epithelial cells with a leukocytic infiltration of the tubules and edema of the glandular epithelium (Dopter and Repaci); an intense, interacinar vascular congestion (Cornil and Ranvier); marked periglandular edema (Jacob,<sup>11</sup> and Reverchon, Worms and Delater); and changes of early necrosis of the glandular epithelium (Rocchi).

#### METHODS, MATERIAL AND PREPARATION OF MATERIAL FOR STUDY

Our studies have been made on *Macacus rhesus* monkeys after infection of the parotid gland following inoculation by cannulation of Stenson's duct.<sup>1</sup> The inoculum in different groups has consisted of 1 to 2 cc. of saliva from patients suffering from epidemic parotitis, 2 cc. of a bacteria-free, saline emulsion of the parotid glands of an infected monkey, and 2 to 3 cc. of a Berkefeld V or N filtrate of a centrifuged emulsion of an infected gland of a monkey, respectively.

Daily gross examinations were made to detect any changes in the periglandular or glandular tissues. It was possible to determine quite readily changes in the size and consistence of the gland by palpation. When the change in size is marked it can be detected by inspection alone. By palpation one is able to note also the presence of edema of the periglandular and subcutaneous tissues, indicated first by a thickening of the skin and then a pitting on pressure when the edema becomes sufficiently increased. The presence of edema marks the height of the reaction to the infectious agent and usually makes its appearance on the 6th or 7th day.

The monkeys forming the basis of this report were killed usually at the height of the reaction by exsanguination after etherization. The initial observations were confirmed postmortem. The skin was

incised and reflected for the examination of the subcutaneous tissues. These were then removed, leaving the capsule of the gland exposed. The alterations, if any, of the capsule and the appearance of the gland through the capsule were noted. The glands were then dissected free, removed to sterile glass containers and weighed, the average weight being 8 gm. They were then sectioned, and strips about 1 to 3 mm. in thickness through the entire gland were prepared for microscopic study. These pieces were taken usually from the middle of the gland so as to include the portion underlying the mandible. During the sectioning, the resistance to cutting, the appearance of the cut surface, and the amount of the oozing serous fluid were noted.

The sections for microscopic study were fixed by a variety of established methods. It was found that tissues fixed by Schaudinn's method were the most satisfactory for studying the cellular changes as well as the infiltrating cells. The secretory granules are dissolved by the fixing fluid with very little or no distortion of the cells, and this eliminates some of the confusion of the picture where the granules are wholly or in part preserved. Tissues fixed in 10 per cent neutral formalin were stained by Levaditi's method for spirochetes.

A routine stain of hematoxylin and eosin was used. Very little additional knowledge has been gained by numerous other staining methods. Mallory's aniline blue connective tissue stain has been used for the demonstration of fibrin.

### CONTROL STUDIES

The monkeys were inoculated by introducing infectious material directly into the gland through Stenson's duct, and an immediate swelling due to the volume of fluid introduced and to trauma occurs, the gland returning to normal within 72 hours. We have felt that some of the alterations found in the glands might be due to mechanical injury thus induced. To learn to what extent injury resulted from the procedure of inoculation, the glands of a normal monkey were prepared in a similar manner and a comparable quantity of the suspension was injected into the parotids of two normal monkeys. Each parotid of one monkey received 2 cc. while each gland of the other monkey received 3 cc. of the suspension.

Daily observations were made on these two monkeys for a period of 7 days. The glands became swollen immediately as the inoculation proceeded. They gradually diminished in size until reaching normal, by 48 hours after the injection, and remained so until the animals were killed.

During exsanguination under ether anesthesia the glands decreased only slightly in size. The subcutaneous tissues on incision were thin, dry and sticky. The capsule of the gland and the subcutaneous tissues were of their usual dullness but the capsule was transparent. Through it the gland appeared pink in color and lobular. No hemorrhages or focal areas of congestion were seen. Each gland of one monkey weighed approximately 2.5 gm., and of the other approximately 3.2 gm. The glands were dry, pasty and sticky, as were likewise the cut surfaces. The lobules, easily distinguished, were compact yet discrete and freely movable. The lymph node enclosed in the gland appeared normal in every respect.

Microscopic examination of these glands revealed no evidence of any inflammatory reaction to any previous or existing irritant. The acini showed no evidence of damage to their epithelial cells. The cell outlines were fairly distinct. The cytoplasm had a uniform granular appearance. The nuclei, which stained rather intensely, were located near the basal attachment of the cell. Here, as in a normal non-inoculated gland, one saw an occasional solitary lymph follicle surrounding partially or completely one of the medium sized ducts. The frequency of such lymph follicles seemed to be somewhat increased in these, in comparison to normal glands. This was the only way in which the glands that received a suspension of normal monkey parotid differed from the glands of normal monkeys. The lymph node embedded in the gland showed no evidence of acute inflammatory reaction. There were a few more large mononuclear phagocytic cells within the sinusoids than are normally seen. Some of these contained phagocytosed particles.

#### THE EXPERIMENTAL DISEASE

(A) *Clinical Evidences:* The experimental disease first manifests itself locally by a slight but detectable enlargement and a moderate increase in the consistence of the parotid on the 3rd to 5th day after inoculation. There is a slight rise in temperature of 1 to 2 days



duration at this time. The leukocytes, which usually increase the day following injection and rapidly decrease the following day, have already reached a definite leukopenic stage at the onset of clinical signs. With the fall of the temperature to normal there is a slight increase in the total circulating leukocytes. The gland shows little or no further change until 24 to 72 hours later when there is a rapid increase in its size. The rapid increase in the size of the gland is followed usually within 12 hours by a rapidly appearing subcutaneous facial edema which soon becomes marked. The tissues over the gland then readily pit on pressure. The beginning of the edema is usually accompanied or slightly preceded by a marked rise in temperature which gradually returns to normal. By the time the edema has reached its height the leukocytes have increased to about their normal count, where they remain with slight fluctuations. Several animals were killed by exsanguination under ether at about or just before it was thought the edema had reached its height in order to obtain the glands for the virus and for the study of the changes present. After bleeding, the gland was found to be slightly but definitely diminished in size. This is interpreted as being due to the collapsing of the dilated and congested vessels of the gland and to the emptying of the large vessels which lie beneath.

(B) *Gross Alterations:* The subcutaneous tissues are thickened by a serous exudate which gives them a glossy and somewhat gelatinous appearance. A considerable amount of serous fluid exudes into the wound as the dissection proceeds. The exposed gland is seen to be enlarged, the surface being oval from its anterior to posterior border with the highest point just posterior to the angle of the jaw, which is the region of a concave area in the normal glands. The capsule of the gland is also thickened and glossy because of the serous exudate. It remains transparent and through it the glandular surface is readily visible. The glandular tissue is of a pale grayish pink color and one can see petechial hemorrhages scattered through it. The number of these petechiae varies widely. In some glands which subsequently prove to be greatly damaged there are no hemorrhages to be seen in the gross, while in others with a much less disseminated process, microscopically, there are numerous visible petechiae. The hemorrhages are occasionally slightly more numerous in the portion of the gland overlying the masseter muscle and along the inferior portion of the gland. However, as seen from the

surface of the gland *in situ* they appear to be about equally scattered in its different portions.

There is a tendency for the gland to become rounded after being dissected free, thus in the receptacle, as *in situ*, the enlargement of the glands appears to be due to its increased thickness. The lateral dimensions may be diminished. The normal gland, on the contrary, lies flat and plastered to the receptacle. The usual weight of the gland and the oozing serous exudate from the gland after removal is about 8 gm., the limits having been 4 and 10.6 gm.

The cut surface of the gland everts, giving it a convexity. The lobules which are of a pale grayish pink color slightly protrude and have convex surfaces. The interlobular spaces are increased in breadth, causing a greater separation of the lobules. The cut surface appears somewhat duller than one might expect of a wet surface but no definite evidence of necrosis in the glandular tissue is to be made out.

(C) *Microscopic Alterations:* Histological sections reveal both diffuse and focal alterations of the gland, neither of which destroys the general architecture (Fig. 1). The diffuse alterations consist in a widening of the interacinar and interlobular spaces by serofibrinous exudate, and a diffuse edema of the acinar cells which often become elevated from the basement membrane. The focal lesions result from destructive changes of the acinar cells, followed by a mononuclear leukocytic infiltration (Fig. 2). A lymphocytic infiltration appears about many of the ducts and later within the injured or destroyed parenchymal foci. These changes are most abundant in the central portions of the lobules and become less marked toward the periphery where the destructive changes are not as far advanced at the height of the swelling as the apparently older lesions in the central portions of the lobules. The hemorrhages noted in the gross examination of the gland are found to be fresh interstitial extravasations of blood frequently but not necessarily associated with the focal areas of necrosis.

These histological observations have been made on the parotid glands of many monkeys during our 2 years of study of the experimental disease. A histological description of a section from any of the glands removed during or shortly after the height of the disease would include all of the essential changes that have been described in the above paragraph. However, during the past ten or more gen-

erations of the virus the focal areas of acute parenchymal destruction have become much more disseminated throughout the gland. Nevertheless, there still remain lobules, either singly or occasionally in groups, which are only slightly affected. The volume of inoculum and the dilution of the emulsion have remained constant. While the clinical evidences of the disease have not seemed to increase in severity, the greater extent of the glandular lesion indicates that the total quantity of virus in the inoculum has increased with successive passage, or that the virus has become more pathogenic for this host.

### HISTOLOGICAL CHANGES

The histological changes in the glands that show the more diffuse dissemination of the areas of acute parenchymal destruction will form the basis of a more detailed description of the histopathology of the experimental disease. The alterations present will be described under the paragraph dealing with the structure of the gland in which the changes occur.

*Acini:* The earliest detected changes, indicative of the formation of the focal degenerative lesions, occur in one or more cells of a single or small group of acini. The cell becomes swollen, the cytoplasm stains paler and loses its normal granulation, often becoming vacuolated as in hydropic degeneration. The nucleus during the early swelling of the cell also seems to be swollen as it stains less deeply and is a little more vesicular. But with the appearance of the more advanced cytoplasmic alterations the nucleus shrinks, becoming pyknotic. It is in this stage of degeneration that the cytoplasmic inclusions to be described later are most commonly found; however, they occur also in cells showing only the edematous swelling. The cell outline now becomes irregular and indistinct, and the cell wall disappears. During these changes the cell often becomes detached, the nucleus tends to break up and the invasion by large mononuclears begins. These recent degenerative changes appear in acini surrounding the more advanced focal areas of necrosis or toward the periphery of the lobule, thus indicating a spread of the disease process (Fig. 3).

The large mononuclear phagocytic cells early infiltrate the degenerating acinus. Occasionally one can be seen within the cytoplasm, but more often they lie along the irregular border of the cell.

They rapidly increase in number within and finally about the acinus. The necrotizing cells are gradually phagocytosed and replaced by the large monuclears which fill the acinar framework and form a discrete inflammatory focus. Later there is an infiltration by lymphocytes admixing with the mononuclear exudate. At this stage it is often difficult to make out the limits or determine the architecture of the remaining framework, for it is obscured by the inflammatory cells. Polymorphonuclears play no part in the cellular infiltration.

Accompanying these focal areas of acute degenerative change there is a more or less diffuse edema of all of the acini, as evidenced by an increase in size of as much as 20 per cent by measurement. The cytoplasm of these cells stains paler and is a little more granular than the normal; the nuclei are usually less deeply stained, slightly more vesicular and placed at more irregular distances from the base. The cells are often elevated from the basement membrane. The nuclei also appear to be less frequent in the acini of the diseased gland than in the normal gland sectioned to the same thickness. This we interpret to be due to the swelling of the cell and not to a disappearance of the nuclei.

Occasionally in the acini showing the advanced degenerative changes there will be one or two cells remaining which escape the necrotizing process. These cells, in sections of glands removed at the height of the disease, are sometimes found in a phase of mitosis.

*Cytoplasmic Inclusions:* The cytoplasmic inclusions referred to occur only in acinar cells and as previously stated appear earliest in cells that show only evidences of edema. Usually in the same acinus, however, there are other cells which show more advanced signs of degenerative change. The inclusion is usually single, rounded, and faintly stained with eosin, though often it retains a tinge of the basophilic stain. It is surrounded by a small but definite halo, and measures about 4 microns in diameter. The inclusion is vacuolated, containing usually two to five vacuoles situated in its periphery (Figs. 4 and 5).

In the cells showing the early changes of necrosis there are similar cytoplasmic inclusions. In these cells there are often two and sometimes three inclusions. Here the inclusions are smaller, measuring 2 to 3 microns in diameter, denser and more polychromatic in staining reaction. They may be slightly oval, with only a single vacuole situated near the periphery. The halo surrounding the inclusion is

narrow but definite. This type of inclusion is seen to exist after considerable advancement of the degenerative process and occasionally persists after complete destruction of the cell or cells of the acinus.

*Interstitial Tissues:* In addition to the previously described degenerative changes and diffuse edema of the acinar cells, the interstitial tissue also undergoes focal and diffuse alterations. The interlobular tissues become more or less diffusely involved by the pouring out of a serofibrinous exudate which causes a marked widening of the interlobular areas. The exudate separates the collagen fibrils of the connective tissue and fine strands or networks of fibrin are seen in the serous exudate when the section is stained by Mallory's aniline blue connective tissue stain (Fig. 7). The degree of inflammatory edema varies in different portions of the gland. It is quite marked around the lobules that show the greatest involvement by focal degeneration of the parenchymal cells and is abundant in the interstitial and subcutaneous tissue. Late in the infection round cells infiltrate the interstitial areas. This infiltration is much more pronounced about the ducts and the vessels. The round cells consist mainly of lymphocytes, large and small, and a few plasma cells. The infiltration about the ducts, mainly the medium sized ducts, becomes more marked late in the disease, and some of the ducts seem then to occupy the central portion of a fairly large secondary lymph follicle. The capsule, though moderately edematous, seldom shows any cellular infiltration.

The interacinar areas are also greatly widened, though here no fibrin is seen in the serous exudate. The interacinar distention is much more evident about the central ducts of the lobules and decreases toward the periphery, where the lesions are less frequent and more recent. The interacinar spaces about the degenerating acini become infiltrated with phagocytic mononuclears and lymphocytes, proceeding from an earlier cellular exudation within the acini (Fig. 6).

The small areas of extravasated blood observed microscopically occasionally involve a degenerating acinus, but more commonly they are confined to the interacinar areas about the degenerating and necrotizing acini.

*Ducts:* The infiltration of round cells into the interstitial tissues surrounding them is the only evidence of the disease process shown by the ducts. The infiltration does not penetrate the inner portion

of their walls nor does the epithelial lining show any change indicative of the disease process.

*Blood Vessels:* In sections from glands removed from monkeys not completely exsanguinated the interacinar vessels remained splotchily congested. The perivascular infiltration of the smaller vessels late in the disease has been described.

*Healing:* The clinical signs (fever, subcutaneous edema over an enlarged parotid) of the experimental disease in the monkey remain at their peak only 12 to 24 hours, after which there is a gradual disappearance in the above named order. This clinical recovery is usually complete within 2 to 7 days after the height of the disease. Several of the monkeys were killed during this period of clinical recovery and at later periods for study of the healing process within the glands. These monkeys furnished the material for the following description.

As the clinical signs diminish the interstitial serofibrinous exudate disappears, along with the early acinar lesions, without fibrous tissue proliferation. The cellular infiltration becomes more diffuse, involving the interlobular as well as interacinar spaces about the accumulations at the focal areas of necrosis. The cells of the interlobular infiltration are scattered and comparatively few in number, except about the ducts where they are accumulating. There is an occasional duct containing desquamated epithelial cells and an infiltration of the basal layer with an occasional mononuclear cell. The large mononuclear cells of the older focal lesions are being replaced by young lymphocytes forming secondary lymph follicles in which there are frequent mitotic figures. Mitotic figures are also making their appearance in the acinar cells remaining in the focal areas (Fig. 9).

As the gland reaches its normal size the diffuse interstitial cellular infiltration gradually diminishes but the periductular infiltration becomes greater. The regeneration of acini soon reaches its height. Repaired acini reappear in the focal accumulations of the lymphocytic infiltration (Fig. 10). The lymphoid cells become more mature, diminish in number and, because of the renewed acini, appear as focal accumulations in the interacinar spaces (Fig. 8).

Where the focal areas of necrosis were small and the cellular infiltration was minimal there is complete restoration of the acini with no remaining cellular infiltration.

The healing process, except for the lymph follicles, is complete 2 weeks from the height of the clinical signs of the disease. These secondary lymph follicles disappear gradually, some probably remaining for long periods, as in 1 case a year after recovery several small lymph follicles were still present.

### DISCUSSION

The foregoing observations on the pathology of experimental mumps in the monkey are based on the study of a large number of specimens obtained at varying intervals during and after the onset of clinical manifestations of the disease. In all instances the infection was initiated by introducing the virus in the form of a suspension or filtrate of a previously infected parotid of the monkey or, as in a few instances, in the saliva from a human case of mumps, directly into the parotid gland of monkeys by injection through Stenson's duct. We have been thus far entirely unsuccessful in attempts to induce clinical mumps experimentally in any other way. The experimental lesion of the parotid may not, therefore, represent exactly that which results from a more indirect infection of the gland as presumably occurs in the human disease.

Unfortunately for the purpose of comparison there is not sufficient knowledge of the pathology of human epidemic parotitis to reconstruct a definite picture of the essential lesion. When one considers, however, the various reports in the literature concerning mumps of the human parotid, it is found that practically all the lesions that we have described as occurring in the monkey have at one time or another been found in man. But notwithstanding the fact that experimental mumps is induced by injecting the virus into the parotid through the excretory duct, it is possible quite clearly to determine the course of events in the gland and to apprehend the essential lesion, and we feel justified in assuming that a similar underlying lesion occurs likewise in the human disease.

It seems quite probable that spontaneous mumps does not cause a uniformly distributed lesion in the affected parotid, for even though the virus be injected directly into the monkey gland through the duct there is a patchy distribution of the ensuing lesion. Therefore, it would seem necessary that the entire gland be studied at a proper period of the infection in order to establish the pathology of the human disease.

In the experimental lesions of monkeys there are an invariable basic lesion and a uniform course of events, so that we have no doubt as to the specificity of the effect of the mumps virus in this animal, although it is of course possible that the virus does not affect the human gland in exactly the same way. The typical mumps lesion in the monkey's parotid results from the immediate effect of the specific virus on the acinar cells focally. The earliest focal lesions are located near the central portions of the lobule, as if there the virus came into most intimate and direct contact with the acinar epithelium, perhaps because acini thus situated may be subjected to greater injury or irritation by the injected material. It is well known that slight injury to susceptible cells renders them more sensitive to the effects of a virus. The early degenerative changes and subsequent necrosis may proceed to completion before there is any local cellular infiltration. This fact is in harmony with the events in most if not all virus lesions, and is a reasonable ground for assuming that the mumps virus is cytotropic — that is, the virus acts in conjunction with a certain type of living cell to reproduce itself and to bring about its specific type of injury in that cell.

Following necrosis of acinar epithelial cells there is an immigration to the spot of large mononuclear cells which phagocytose and remove the débris. Remaining acinar epithelial cells and perhaps terminal duct cells regenerate, restoring for the most part the acinar unit, without scarring.

It is not easy to understand how the extensive edema of the gland and surrounding tissue is brought about, but presumably there is a diffuse injury to the general vascular bed, possibly by a toxin or by products of cellular degeneration. The hemorrhagic foci are evidences of the greatest effect of this sort on the blood vessels.

After the initial, more centrally located, lesions have manifested themselves there is a secondary spread of virus through the gland; and a second crop of focal lesions more distally located make their appearance. Therefore when the parotid is removed at the height of the disease, lesions at various stages in their development, from those in which there is complete necrosis to others where degeneration is just appearing, may be found and the evolution of the essential lesion is brought to view.

Regarding the specificity of the cellular inclusions that we have described we can at the present time only express an opinion. Our



experience of cellular inclusions in other virus diseases leads us to believe that these are specific, but we realize the difficulties of effectively substantiating this point of view. Anyone, however, may satisfy himself, we feel, as to the primary effect of the virus in relation to the epithelial cell of the parotid acinus.

Well developed clinical mumps in the monkey depends on the extent of infection in the gland. Unless the focal lesions are rather widely distributed there is relatively little subcutaneous edema, and a focal infection sufficient to induce immunity may proceed to healing without evidence of clinical mumps.

It seems probable that the initial rise in temperature, slight glandular enlargement and beginning leukopenia coincides with the development of the first crop of focal lesions, and the secondary febrile period associated with great glandular edema is associated with a secondary spread of virus and focal lesions more generally throughout the gland.

#### SUMMARY AND CONCLUSIONS

1. A study of the histopathology of experimental mumps in *Macacus rhesus* monkeys is reported.
2. The literature concerning the lesions of human mumps is reviewed.
3. In experimental mumps the essential lesion is the result of a specific action of mumps virus on acinar epithelial cells in focal areas resulting in degeneration and necrosis of the affected cells.
4. The inflammatory cellular response is secondary to the specific injury and consists of an infiltration of the area by mononuclear phagocytic cells and later by various types of lymphocytes. Polymorphonuclear leukocytes play no part in the reaction.
5. There is a secondary dissemination of lesions within the infected gland.
6. The edema is probably dependent on a diffuse vascular injury secondary to the focal parenchymal lesions.
7. Healing takes place by removal of the debris and regeneration of acinar epithelium which restores the gland without scarring.
8. Inclusions are described in the affected epithelial cells, and they are interpreted as specific.
9. It is concluded that similar lesions in all probability underlie the pathology of mumps in the human.

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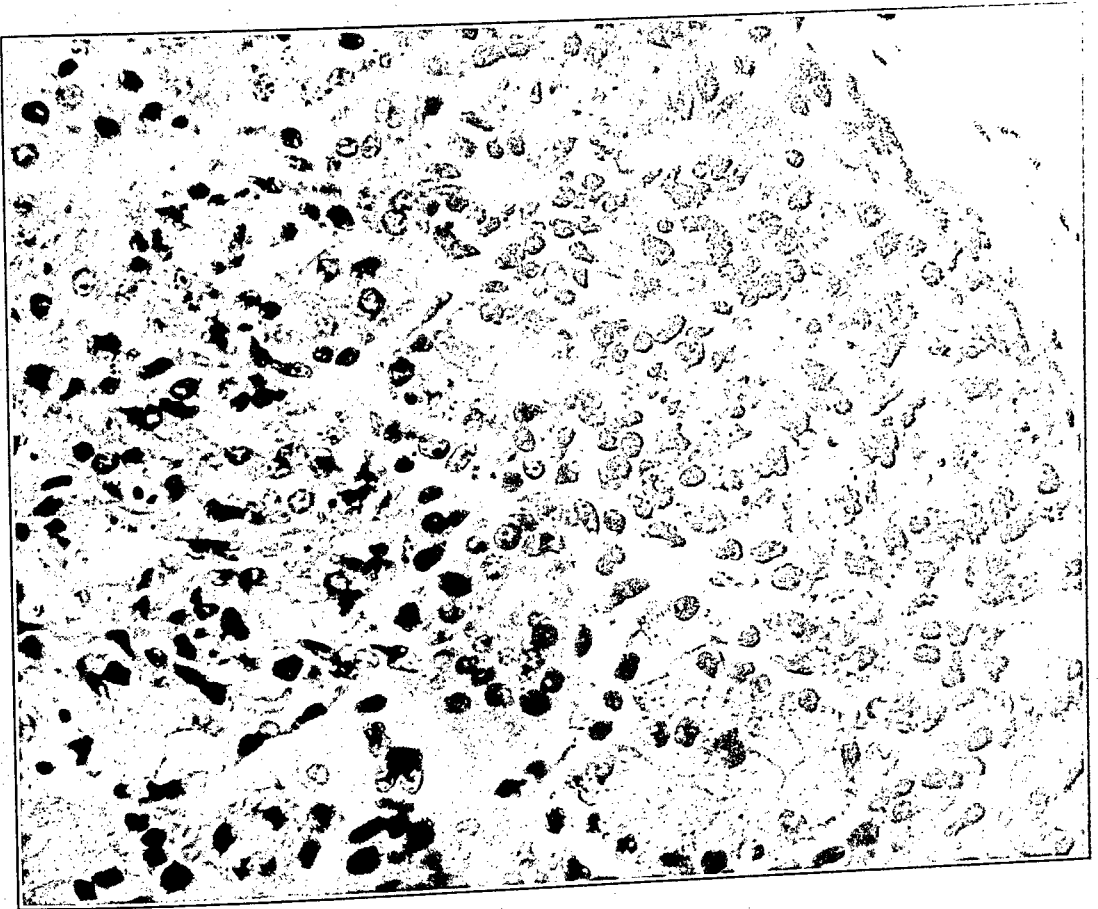
## DESCRIPTION OF PLATES

## PLATE 95

- FIG. 1. Section of parotid of monkey receiving the twenty-ninth transfer of virus in series. Gland removed during height of clinical reaction. Focal necrosis, cellular infiltration and interstitial edema are shown.  $\times 55$ .
- FIG. 2. A higher magnification of a focal area of necrosis in which there has been a moderate infiltration by large mononuclear phagocytic cells and lymphocytes.  $\times 450$ .



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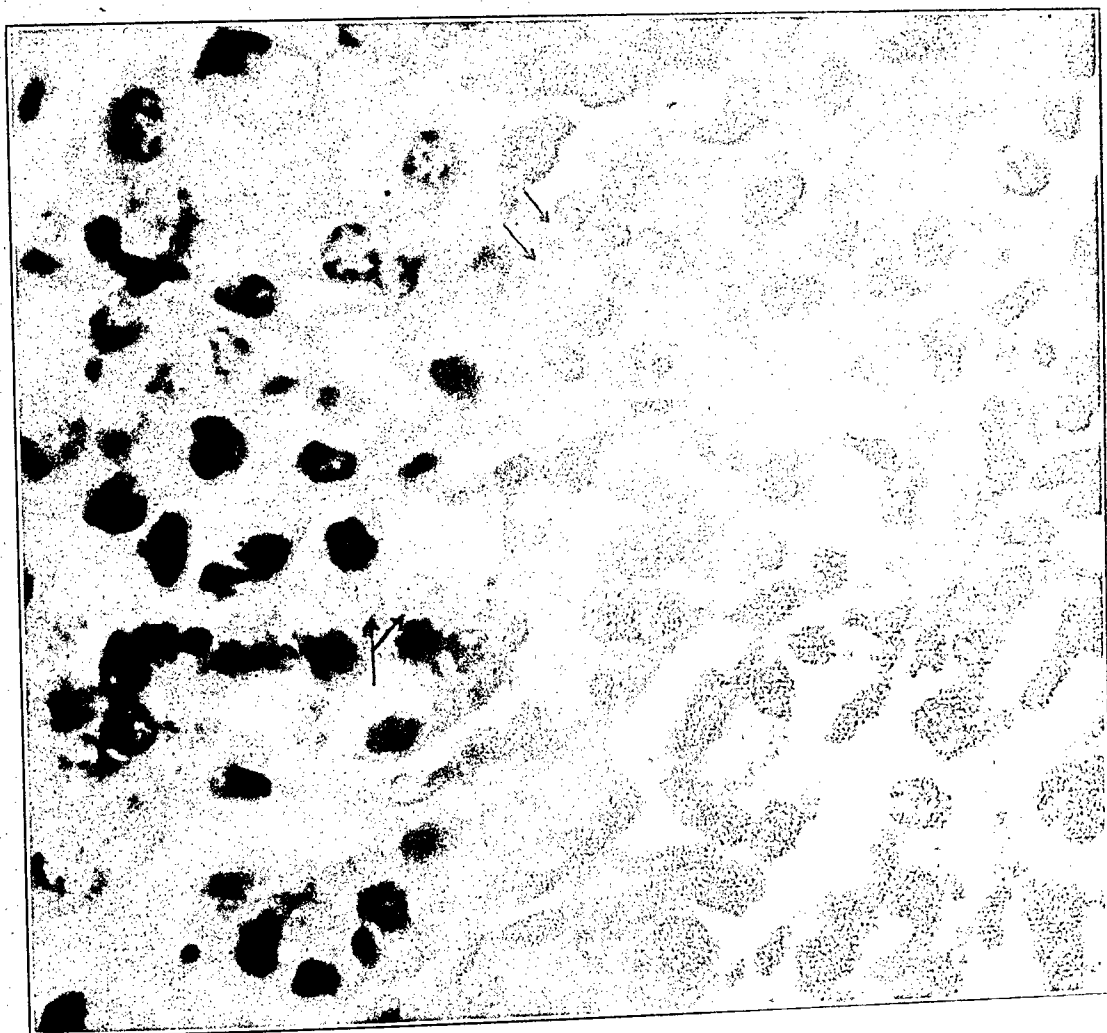


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PLATE 96

FIG. 3. A focal area of very early necrosis preceding cellular infiltration. Arrows mark cytoplasmic bodies seen in the areas of early necrosis.  $\times 1100$ .

FIGS. 4 and 5. Higher magnification of the cytoplasmic inclusions. Note the vacuoles within the inclusions and the surrounding halo.  $\times 2000$ .



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4



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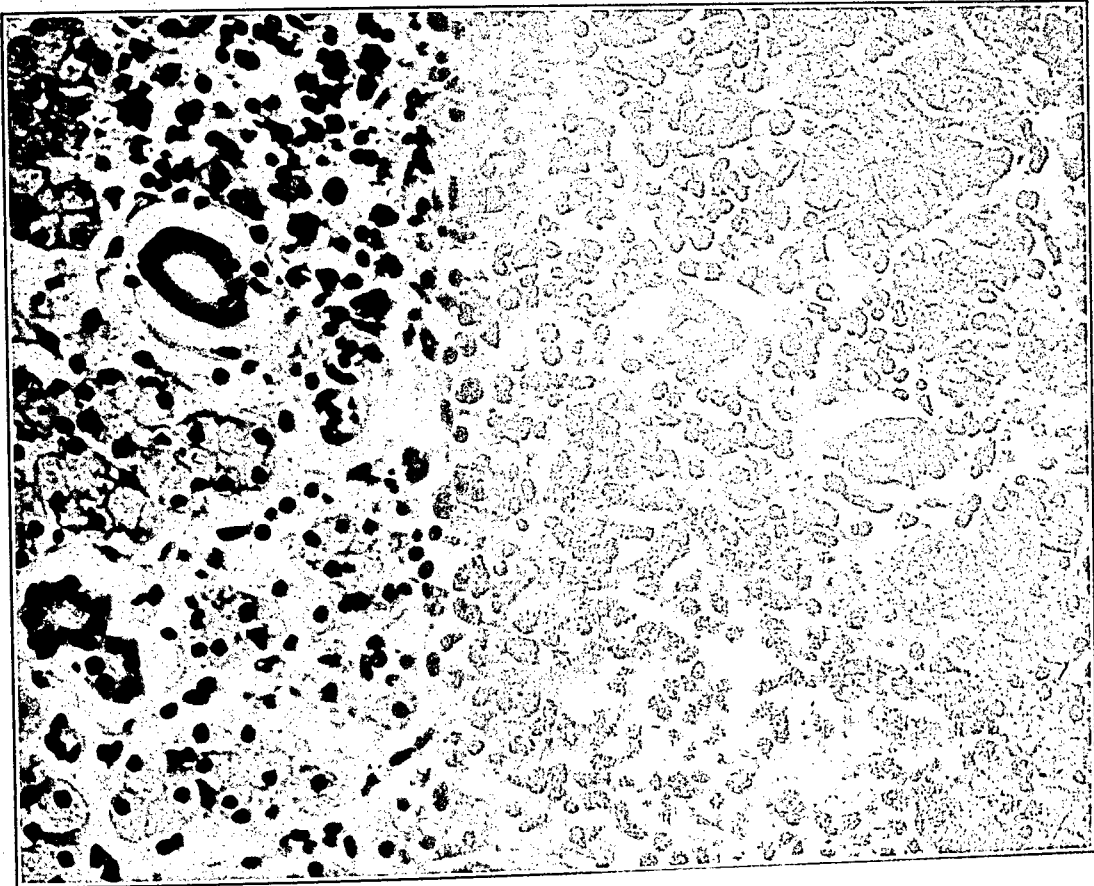
Johnson and Goodpasture

Experimental Mumps in the Monkey

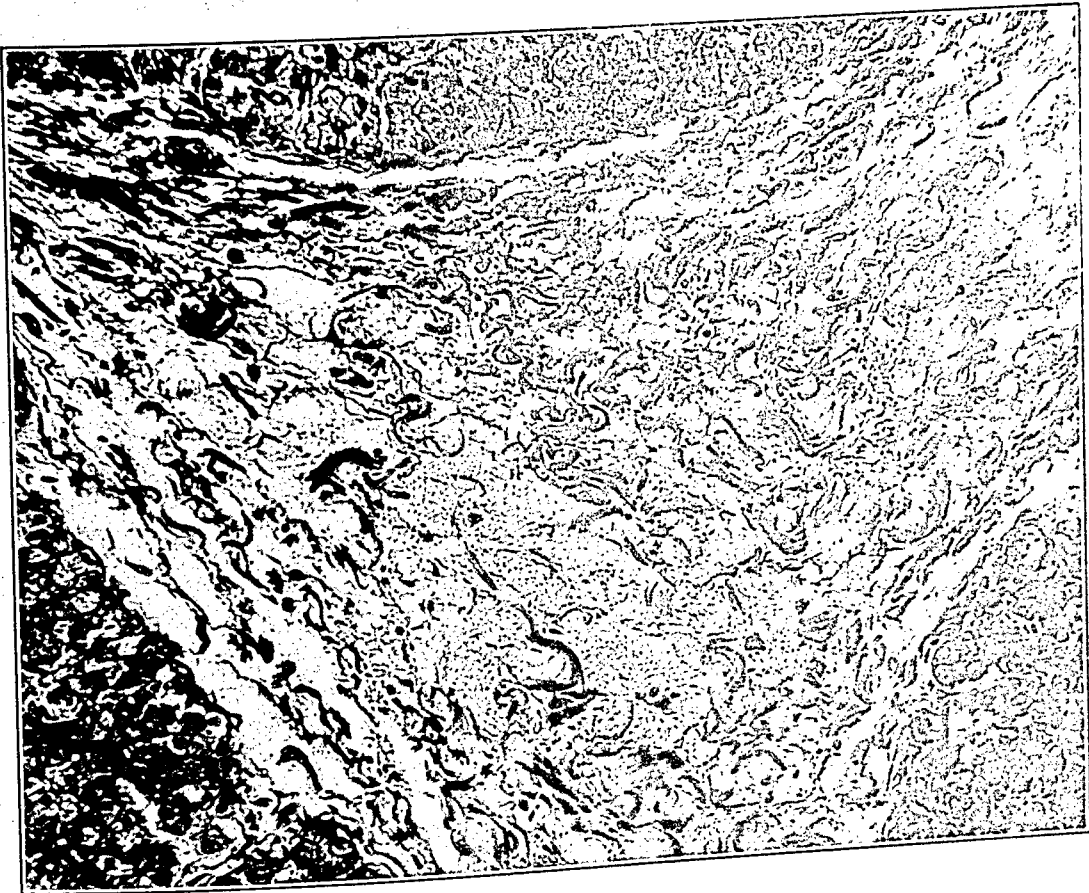
PLATE 97

FIG. 6. Late area of focal degeneration in which the predominating infiltrating cell is now the lymphocyte.  $\times 300$ .

FIG. 7. An area of interlobular connective tissue showing the interstitial edema and strands of fibrin. Mallory's aniline blue connective tissue stain.  $\times 200$ .



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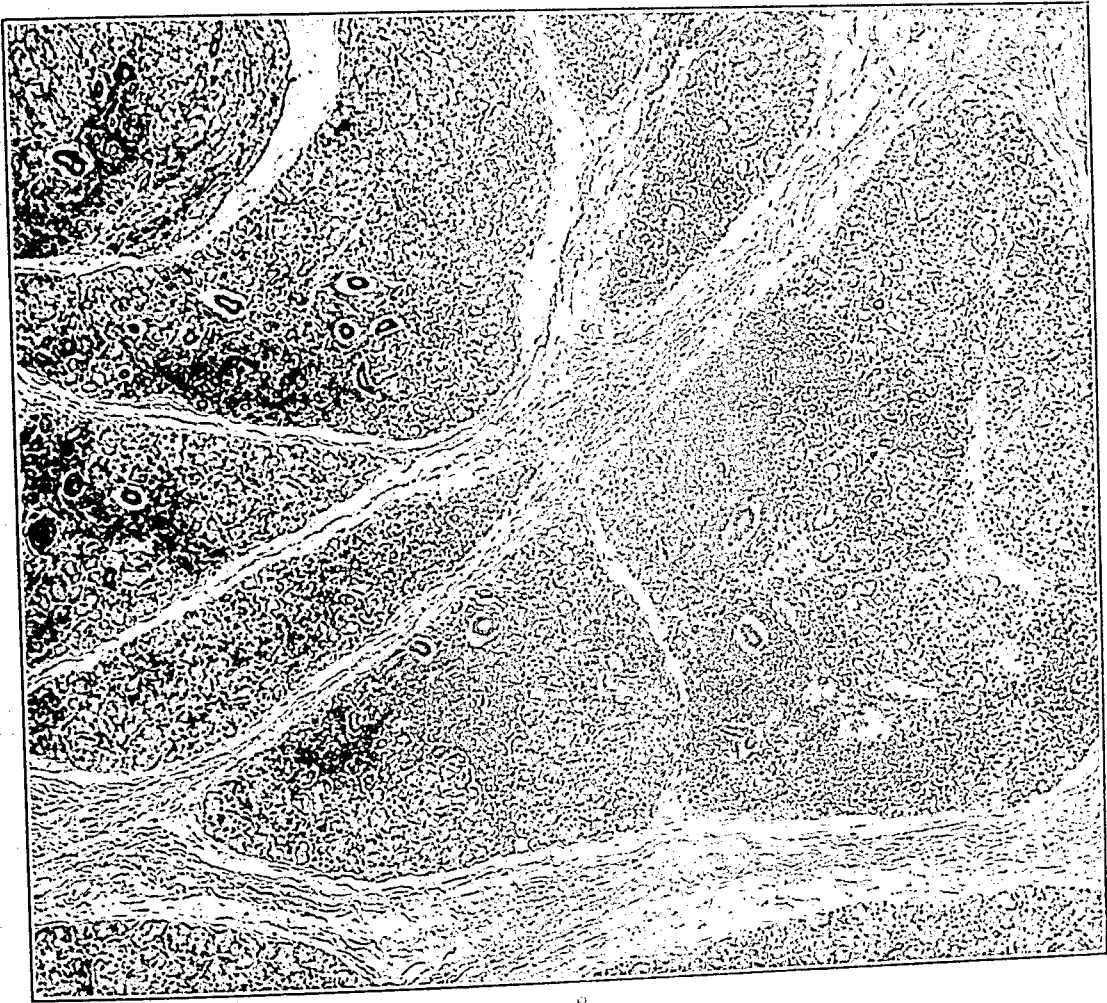
PLATE 98

FIG. 8. Section of a gland removed 2 weeks after height of reaction showing the focal accumulations of the lymphocytes, mainly about the ducts. Compare with Figure 1.  $\times 55$ .

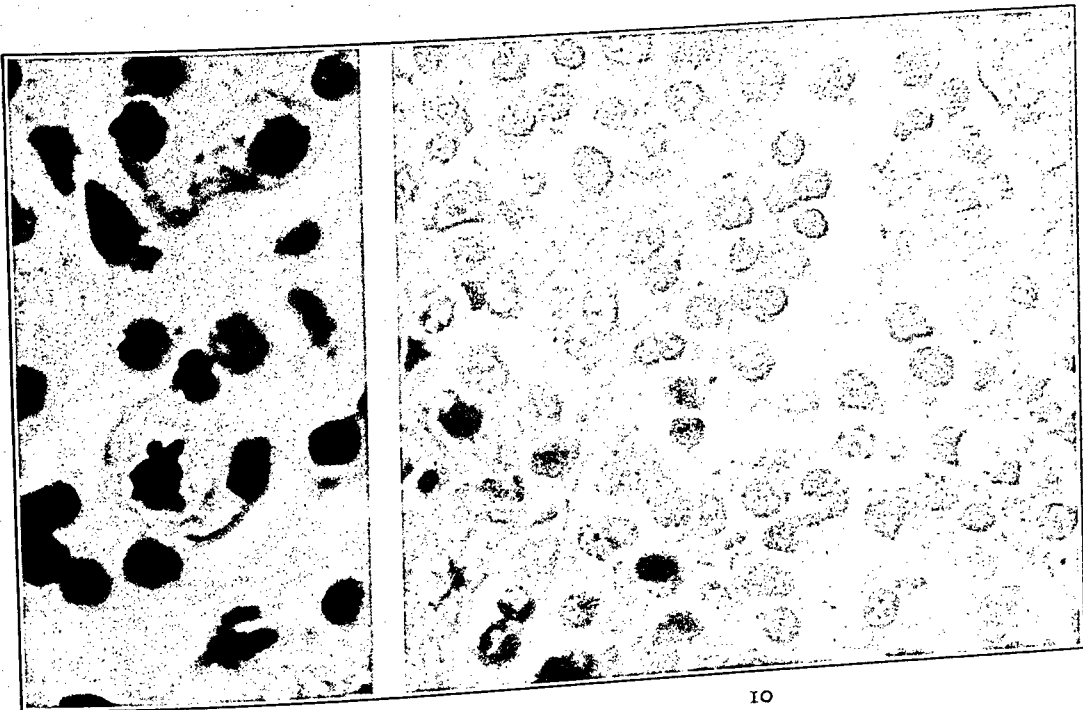
FIG. 9. A mitotic figure of an acinar cell in one of the areas of healing.  $\times 1400$ .

FIG. 10. Type cell in interstitial exudate of healing gland.  $\times 750$ .

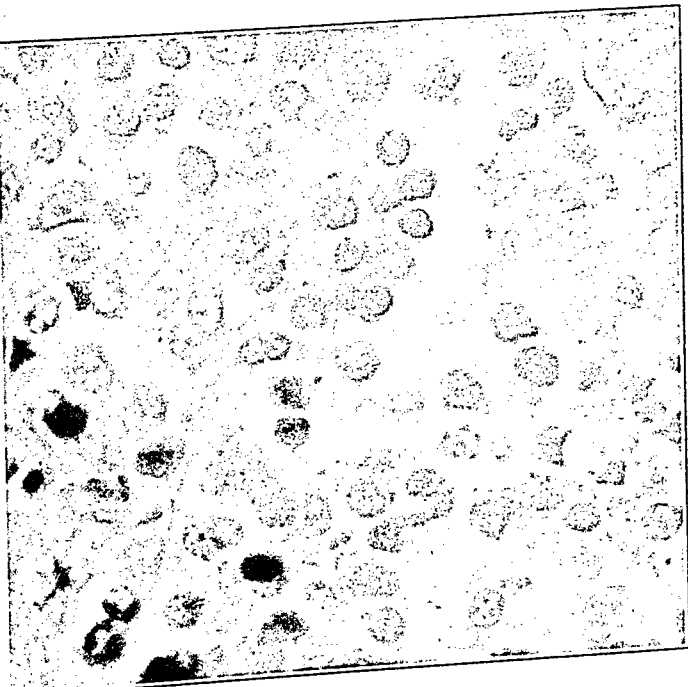




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COMPARISON OF THE BEHAVIOR OF A NEUROTETICULAR  
AND A DERMAL STRAIN OF VACCINE VIRUS IN THE  
CHORIO-ALLANTOIC MEMBRANE OF THE  
CHICK EMBRYO \*

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A method for the production of vaccine for antismallpox prophylaxis in man by cultivating the virus in the chorio-allantoic membranes of chick embryos has recently been described by Goodpasture and Buddingh.<sup>1</sup> From the experiments reported it appears that vaccinia virus may be propagated through an indefinite series of transfers in the membrane without the loss of any of the essential qualities of the original strain of dermal vaccine employed for the initial inoculation. The virulence of the vaccine cultivated by this method is soon established at a somewhat lower level for the rabbit and for man than that of the original calf strain, as evidenced by the character of the lesions induced by it. After the virus has been carried through 6 to 8 generations in the membrane of the chick embryo, no variations have been observed in its effects over a 3 year period of continuous cultivation through 175 successive generations, during which time no intervening passage was made in any mammalian host.

The lesions induced in susceptible persons by vaccine from the 175th generation in the chick membrane are quite similar in kind and intensity to those from the 6 generations; and more than 1000 vaccinations performed on children and adults with vaccine prepared from the 100th generation proved in every way satisfactory from the standpoint of the evolution of the lesion and in the establishment of immunity to subsequent (1 year) vaccinations with a potent calf vaccine.

From a practical standpoint, as well as theoretically, it is of great importance in estimating the quality of a particular strain of vaccine virus to determine to what extent and in what manner it may be altered by continuous cultivation in the membranes of develop-

\* Aided by grants from the Divisions of International Health, and of Medical Sciences, Rockefeller Foundation.

Received for publication December 14, 1935.

ing chicks. In a uniform living medium of this kind, which can be kept free from bacterial contamination, apparently standard conditions may be obtained for the investigation of this problem.

That vaccinia virus can be cultivated on tissues other than the skin is well known. Thus Noguchi<sup>2</sup> established a method of obtaining a pure culture of the virus in the rabbit testicle. Although he did not find that virus propagated by this method was modified in its virulence for the skin,<sup>3</sup> other observers, Hach<sup>4</sup> and Arnold,<sup>5</sup> have found that testicular virus possessed qualities that produced severe reactions and widespread necrosis.

Levaditi and Nicolau<sup>6,7</sup> adapted a strain of vaccine virus to nervous tissue by continuous propagation in the rabbit brain. The hemorrhagic and necrotizing properties of this strain of neurovaccine are well recognized by most investigators in this field.

Since Steinhardt and her colleagues<sup>8</sup> demonstrated that vaccine virus could be propagated in tissue cultures, numerous investigators have, with many modifications, used this method for the study of its effect on the maintenance or modification of the characteristics of the virus. When propagated in this manner, Haagen,<sup>9</sup> using a rabbit testicular vaccine virus which was passed through 37 generations in rabbit testicle tissue cultures, found no increase or diminution in its virulence. Later, Haagen, Gildemeister and Crodel<sup>10</sup> reported that another strain cultivated in this manner acquired a distinct capacity to produce hemorrhagic and necrotizing lesions when injected subcutaneously into the rabbit skin, although no increase in its virulence titer took place during 21 passages. Eagles and McClean<sup>11</sup> also found that growth of both dermal and testicular strains of the virus in tissue cultures was not accompanied with any essential change in their characteristics when inoculated in the rabbit skin.

Rivers,<sup>12</sup> and Li and Rivers,<sup>13</sup> using a dermal and neurovaccine, respectively, in minced chick embryo suspended in Tyrode's solution, were successful in propagating these strains through numerous passages without observing any change in their essential characteristics, although a definite increase in titratable infectivity took place.

Nauck and Paschen<sup>14,15</sup> also found that the characteristics of a humanized dermal strain of vaccine virus propagated in rabbit testicle tissue cultures were maintained even though a great increase in the titratable infectivity of the virus took place in the course of 70 generations.

In the light of these several investigations it was of special interest to know whether or not it could be definitely established that cultivation of vaccine virus in the membranes of the developing chick could be expected to maintain or alter the essential characteristics of a particular strain of virus.

That the dermal and neurotrophic strains of vaccine virus have clear-cut and distinct properties, as evidenced by the hemorrhagic and necrotizing action of the neurovaccine in contradistinction to the dermal vaccine, is well recognized. A satisfactory explanation for these differences has until the present not been offered. Since the vaccinal lesions in the chorio-allantoic membrane lend themselves remarkably well to a histopathological study, an attempt to find a possible explanation for such a variation was made in the following investigation. A practical side of the problem involved the question whether or not original desirable qualities of a particular strain for human immunization would be suitably maintained by continuous cultivation in the membranes of chick embryos without intervening mammalian passage.

Two distinct strains of vaccinia virus, one a neurotesticular strain,\* and the other a calf dermal strain,† were used. The attribute used for the basis of comparison was the constant hemorrhagic character of the lesions produced in the rabbit skin by the neurotesticular virus. This characteristic is well described by Levaditi and Nicolau,<sup>6</sup> and well recognized by numerous other investigators. Propagation of the strain of neurotesticular vaccine in the chorio-allantoic membrane of the chick embryo was undertaken for the purpose of comparing its behavior with that of the dermal strain. Preliminary observations had indicated that the membranal lesion produced by the neurotesticular virus could likewise be distinguished from the lesion produced by the dermal vaccine by its hemorrhagic character. This distinction also characterized the vaccinal lesion produced in the rabbit skin by the neurotesticular vaccine after it had been propagated in the chick membrane through several generations.

A histological study, in which the vaccinal lesions produced by the dermal and neurotesticular strains of vaccine in both the chick

\* The neurotesticular strain was kindly supplied to us by Dr. T. M. Rivers of the Rockefeller Institute. It was a strain of Levaditi's neurovaccine which has been maintained by passage through the rabbit testicle.

† Obtained from the New York City Health Board Laboratories.

membrane and the rabbit skin were compared, was made to determine whether or not the apparent differences in these strains were reflected in the cellular structure of the lesions. In this manner some conclusion might be reached regarding the reasons underlying the variances in virulence and pathogenicity of these two strains.

### EXPERIMENTAL

The strain of neurotesticular virus was propagated in pure culture through 50 generations in the chick membrane during a period of 10 months. Transfers, when made, were at intervals of 48 hours. Small bits of bacteria-free, infected membrane were used to inoculate 14 day embryos, which were then incubated at 37° C for 2 days. A dermal strain of virus was propagated through 50 generations in the same manner. The technique used was the coverslip method described by Goodpasture and Buddingh.<sup>1</sup>

The membranal lesions were carefully observed to detect any macroscopic changes which might take place. Special attention was directed to the hemorrhagic character of the lesions from the neurotesticular vaccine. Smears from the lesions were made regularly and stained by the Morosow method to demonstrate Paschen bodies.

At the 10th, 20th, 30th, 40th and 50th generations the lesions from at least 10 embryos were collected and ground in a sterile mortar. To each part of the ground pulp, 4 parts of sterile 50 per cent glycerine were added. This vaccine was inoculated into the scarified rabbit skin. The lesions developing from both the dermal and the neurotesticular strains of vaccine were observed and compared.

At the 20th and 50th generations sections were taken from the 4th day lesion in the rabbit skin and fixed for histological study. Forty-eight and 72 hour lesions in the chick membrane were also removed at regular intervals and fixed for histological study.

### THE EFFECT ON VACCINE VIRUS OF CONTINUOUS SERIAL TRANSFER IN THE CHICK MEMBRANE

The 48 hour membranal lesion from the neurotesticular vaccine is characterized by a slight thickening and opacity of the inoculated area. Scattered throughout are numerous small, discrete, pock-like elevations. A gray opacity radiates from the periphery of the lesion for some distance along the blood vessels. This thickening and

opacity is much more marked in the lesions from the dermal strain. In the latter the pock-like elevations are much larger and more numerous, and thick plaques of cream colored exudate cover the infected area. The latter lesion is much more definitely circumscribed and does not extend along the blood vessels to as great an extent as the former.

The sharpest contrast between the lesions from the two strains of virus is in the areas of hemorrhage produced by the neurotesticular strain. These vary in size in the membranes from small petechiae to large patches of hemorrhage. Both *in situ* and when removed from the embryo, the lesion from the neurotesticular vaccine is easily distinguished from the dermal strain by its bloody appearance.

When these two strains of vaccine are inoculated into the scarified rabbit skin the same striking difference appears. Both produce by the 3rd day a characteristic papular vaccinal lesion which becomes pustular on the 4th day. By the 5th or 6th day the reaction has reached its height; crust formation has taken place and the lesion then gradually subsides. In the lesions from the neurotesticular vaccine the papules are surrounded by a blue or violet zone of discoloration. There is an intense congestion of the papules and the subcutaneous tissue is involved much more extensively. By the 4th or 5th day the pustules have black necrotic centers. The crusts which develop are dark brown to black. They are much thicker and more deeply seated than the crusts on the lesions from the dermal strain. Separation of the crusts in the neurotesticular lesion proceeds much more slowly and a much deeper scarring occurs than that following the dermovaccinal lesion.

These characteristics have sharply distinguished the behavior of these two strains of virus throughout the entire investigation. The hemorrhagic character of the membranal lesion has persisted without any noticeable alteration after propagation through 50 generations in the chick membrane.

If the lesions produced in the rabbit by the original neurotesticular virus are compared with those produced by the same strain after it has been propagated through as few as 5 or 6 passages on the chick membrane there is a difference in the extent of the injury and in the amount of reaction which takes place in the infected rabbit skin. The neurotesticular virus produces a relatively profound injury which is evidenced by the intense induration and edema of the

subcutaneous tissues and the subsequent marked necrosis of the overlying skin. After 5 or 6 passages in the membrane there is a distinct diminution in these effects. There is only moderate induration of the subcutaneous tissues and the subsequent necrosis is less extensive. This diminution in the virulence of the virus is effected rather quickly after it has been propagated in the membrane. However, careful observation of these characteristics of the lesions in the rabbit convince us that once this diminution in virulence has been effected there is little further change. Quite a uniform level of virulence has been maintained throughout the 50 generations of the neurotesticular virus in the embryonic membrane.

The same observation was made with regard to the dermal strain of vaccine. The original calf vaccine also produces a much more extensive reaction in the rabbit skin in the way of subcutaneous induration and more extensive necrosis than does the same strain after it has been propagated in the chick membrane through 5 or 6 passages. After the lower level of virulence is once established it is maintained at this point, and throughout the succeeding 50 generations no further change has been observed.

On the other hand, the property of the neurotesticular strain that is responsible for the hemorrhagic lesion in the rabbit skin and on the membrane is not altered by propagation in the membrane. The appearance of the lesions produced by the virus after being passed through 50 generations in the membrane was in no way different from the lesions produced by the original strain in respect to the character and extent of the hemorrhage in the inoculated rabbit skin or in the chick membrane. Cultivation in the chick membrane evidently does not affect the neurotesticular virus in its ability to produce the pathological changes in the blood vessels that are responsible for the hemorrhage and necrosis.

### HISTOLOGY

Paraffin sections of the lesions from the membrane and the rabbit skin which had been fixed in Zenker's solution were made. Routine staining in hematoxylin and eosin was done for the study of the general structure of the lesion. Guarnieri bodies were demonstrated by treating the sections for 30 seconds with a 0.4 per cent solution of potassium permanganate, staining in a 2 per cent solution of acid



fuchsin for 10 to 20 minutes and counterstaining in a 1 per cent solution of methylene blue, then differentiating in 95 per cent alcohol, absolute alcohol, clearing in xylol and mounting in cedar oil.

The 48 hour lesion in the membrane was found to be best suited for comparative study. At this stage the membrane infected with the dermal strain is greatly thickened on account of edema, inflammatory exudate and cellular hyperplasia. In the older areas of involvement the ectodermal epithelium has become entirely necrotic. A heavy infiltration of inflammatory cellular exudate fills these areas and the entire adjacent mesodermal layer. The exudate consists mainly of polymorphonuclear leukocytes, numerous large round mononuclears and a few red blood cells. There is an intense edema of the entire mesodermal layer. The blood vessels are congested. Proliferation of the cells in the entodermal layer has taken place in the areas where the lesion is most advanced. At the advancing margin of the lesion the epithelial cells of the ectoderm have proliferated extensively and degenerative changes have taken place. Many of the cells have become greatly swollen and contain large vacuoles. The nuclei have become pale and irregular in outline. In these areas formation of typical vesicles is frequently encountered. Directly beneath in the mesoderm many fibroblasts have proliferated and the walls of the blood vessels appear greatly thickened.

The 48 hour lesion in the membrane from the neurotesticular virus is characterized by less thickening and swelling. The necrotic areas in the ectodermal epithelium are much smaller and the inflammatory exudate is less abundant and extensive. Scattered throughout the mesoderm and frequently among the cells of the ectodermal layer are numerous areas of hemorrhage. These can be seen extending from the blood vessels, chiefly the veins, or as free collections of red blood cells in the mesodermal tissue. Such areas of hemorrhage in many instances show no evidence of inflammatory changes in their vicinity. Around the blood vessels great proliferation of fibroblasts has taken place so that their walls appear to be several times thicker than normal. Within many of the veins a margination of thrombocytes is seen along the entire inner wall of the vessel. The endothelial lining is remarkably irregular and the individual cells are swollen and distorted. Throughout the mesoderm fibroblasts have proliferated in great numbers and are frequently encountered as

small masses. Along the margin of the lesion the ectodermal epithelium has undergone less proliferation than in the lesion from the dermal strain. Formation of vesicles is also frequently seen. In areas where the lesion is most advanced the entodermal epithelium has undergone rapid proliferation.

The 96 hour vaccinal lesion in the rabbit skin can be briefly described and compared. The dermal strain of vaccine produces changes that are most marked in the dermis and epidermis, while the neurotesticular virus involves the subcutaneous tissue to a considerable extent. In both types of lesions necrosis of the epidermal epithelium has taken place and these areas are heavily infiltrated with a polymorphonuclear exudate which has spread over the surrounding area and has formed a crust of necrotic leukocytes and cellular débris. Along the edges of the lesion the epithelial cells are hyperplastic and show degenerative changes. Many of them are swollen and contain large vacuoles. The nuclei are pale and irregular in outline. There are also hyperplastic and degenerative changes in the epithelial cells of the hair follicles.

While these degenerative and inflammatory processes involve only the dermis and epidermis in the lesions from the dermal strain, the subcutaneous tissue in the lesions from the neurotesticular strain show many evidences of involvement. There is considerable edema present. Large collections of inflammatory exudate are present around the blood vessels. Many of the vessels are filled with fibrin thrombi. Some of the vessels can be seen to have a collection of blood platelets around their inner margin. The endothelial lining is irregular and the cells are greatly swollen. Hemorrhages into the areas surrounding these vessels are frequent. This marked involvement of the blood vessels by the neurotesticular vaccine is quite striking and does not seem to take place in the lesions from the dermal strain.

#### GUARNIERI BODIES

In sections stained to demonstrate the cytoplasmic inclusions of vaccinia there is a marked difference in the distribution of these structures in the lesions produced by the two strains of virus. In the membranal lesions produced by the dermal strain inclusions are found chiefly in the ectodermal epithelial cells in the areas of pro-

liferation at the advancing edge of the lesion. They are present in varying shapes and sizes. They may be seen as long thread-like processes partly surrounding the nucleus, or as triangular or irregularly rounded structures. They range in size from small granules to masses that fill almost the entire cytoplasm of the cell. Many of them are surrounded by a clear zone. In the vicinity of the areas in the ectoderm that are most heavily infected, a few fibroblasts of the mesodermal layer may also be found to contain inclusion bodies, but these are not generally distributed throughout the mesoderm. In the blood vessels directly adjacent to the heavily infected areas in the ectoderm an occasional endothelial cell may be found to contain an inclusion body.

In the membranal lesion from the neurotesticular strain the inclusion bodies are found not only in the ectodermal epithelium but are present in the majority of the fibroblasts throughout the entire mesodermal layer. In areas where fibroblasts are found in small groups, each cell is seen to be filled with the inclusion material. Where proliferation of the fibroblasts has taken place around the blood vessels, inclusion bodies in these cells are particularly numerous and the cells appear to be diffusely filled with this material. In the blood vessels where margination of blood platelets has taken place practically every endothelial cell can be seen to contain more or less included material. Inclusion bodies can be found in some of the endothelial cells of most of the blood vessels in the lesion.

It appears that the neurotesticular virus involves the mesodermal structures of the membrane to as great an extent as it does the ectodermal epithelium; while the dermal vaccine has a predilection chiefly for ectodermal epithelium.

Inclusion bodies could occasionally be found in the epithelial cells of the entodermal layer in lesions from the neurotesticular strain, but were not demonstrable in the cells of this layer in lesions from the dermal strain.

In the lesions in the rabbit skin, cytoplasmic inclusions were clearly demonstrable in the epithelial cells of the Malpighian layer at the edge of the lesion and in the epithelial cells of the hair follicles in both types of vaccinal lesions. They were not observed in the fibrous tissue or the blood vessel endothelium in the subcutaneous tissue of the lesions produced by the neurotesticular virus.

## PASCHEN CORPUSCLES

Smears made from the membranal lesions stained by the Morosow method for the demonstration of Paschen corpuscles brought out no distinctions between the two strains of virus. There appeared to be no demonstrable difference in morphology and the 48 hour lesions from each strain always contained these bodies in enormous numbers.

## DISCUSSION

A few conclusions may be drawn as the results of these experiments in regard to the effect on vaccine virus of continuous cultivation in the chick membrane. As has been shown by the numerous other investigators already referred to, cultivation of the virus in tissue cultures, although usually attended by an increase in the titratable infectivity of the virus, in most instances is not accompanied by any change in the essential characteristics of the virus. Cultivation of the virus in the living chick membranes results in no decrease in the original titratable infectivity of the virus and in some instances a definite increase is observed. There is also no alteration observable in the basic characteristics of a particular strain of virus as the result of this method of cultivation.

Definite indications of a clear-cut histopathological distinction accounting for the difference in behavior of the neurotesticular and dermal strain of virus have been found. The neurotesticular strain of virus, besides its property of affecting specifically the epithelial cells of the ectodermal layer of the membrane, has a greater predilection for involving the cells of the mesodermal layer and more particularly the endothelial cells of the blood vessels. The injury thus produced with the resulting thrombosis and destruction of blood vessel walls is sufficient to account for the hemorrhagic and necrotic characteristics of the lesion produced by this strain of virus.

The dermal strain, on the other hand, evidently has an affinity more restricted to the epithelial cells of the ectodermal layer. This difference in the two strains of virus is a distinct one and is clearly demonstrable in both the chick membrane and the rabbit skin.

The heightened predilection of the neurotesticular strain of virus for structures of mesodermal origin may possibly have been acquired by its having been continuously cultivated over long periods of time in internal organs away from dermal epithelium.

The great variation in effects produced by different strains of presumably dermal vaccines is known to many investigators. The possibility of different tissues such as brain or testicle acting as a selective medium whereby a certain predominant element in a given strain may be separated and maintained is another theoretical explanation for the development of such distinct strains as the neurotrophic and testicular strains of the virus which must be considered.

Continuous propagation of the virus in the chick membrane does not appear to alter the pathogenicity of the virus. This is evidenced by its ability to involve particular types of cells and to produce a characteristic pathological picture. After 50 transfers in the membrane over a period of 10 months the neurotesticular virus still induces in the rabbit skin and in the chick membrane a lesion that does not vary in its hemorrhagic characteristics from those induced by virus from the earlier passages.

It is evident also that this method of culture does affect the virulence of the virus for the rabbit, as shown by the diminution of the intensity of the injury and the amount of reaction in the lesions produced by virus which has been propagated in the membrane through a few passages, as compared with the lesion produced by the original rabbit testicular or dermal calf strain. Cultivation of the virus in the membrane rather quickly brings about this diminution in virulence to a definite level, after which little further change in this property has been observed through 50 successive passages.

The same phenomenon has been observed in another strain of dermal vaccine which has been cultivated in the chick membrane for the past 3 years through 175 generations without intervening mammalian passage. This strain has been inoculated on rabbits at regular intervals from the 6th to the 175th generation and also on a large number of human subjects. The slight diminution in virulence both for the rabbit and for man, as compared with the original calf vaccine, was present at the 6th passage in the membrane. Since that time little if any further change has been noted and the lesions produced by the virus have been markedly uniform in character.

The fact that once this slightly lower level of virulence of the virus is established by cultivation in the membrane and is thereafter seemingly maintained indefinitely is of obvious practical importance. A vaccinia virus which can be maintained at a stable level of virulence without the necessity of intervening animal passages which

are likely to induce unforeseen and undesirable changes in both its pathogenicity and its virulence is highly desirable in the field of human prophylaxis.

Our observations as to the effect on the immunizing property of vaccine virus of cultivation in the chick membrane have been reported elsewhere.<sup>1</sup> A vaccine prepared from cultures of virus passed through 100 generations in the membrane has produced in humans a substantial immunity as tested by revaccinations with a potent calf vaccine after a period of 1 year. Further observations on this property of the virus will have to be made as time goes on.

The above observations seem to us to confirm our contentions that the chorio-allantoic membrane of the chick embryo may be regarded as a stable medium for the propagation of vaccinia virus. They have also given some insight into the problem of the underlying reasons for the difference in pathogenicity of two distinct strains of the virus. Because most, if not all, viruses are cytotrophic — that is they seem to require an intracellular environment for reproduction — it is interesting to note that in two variants of the same species of virus their essential differences seem to be based on their own peculiar affinity for definite types of cells.

#### SUMMARY AND CONCLUSIONS

1. A neurotesticular strain of vaccine virus produces a hemorrhagic type of lesion in the chorio-allantoic membrane of the chick embryo and in the rabbit skin.
2. The hemorrhagic quality of the lesion produced by the neurotesticular vaccine has been maintained through 50 passages in serial transfer in the chick membranes.
3. A comparative histological study of the lesions produced by a dermal and a neurotesticular strain of vaccine virus in the membranes of the chick embryo and in the rabbit skin has shown that the neurotesticular strain is characterized by a special affinity for mesodermal structures, especially the endothelial cells of the blood vessels.
4. During cultivation of a strain of vaccine virus in the chorio-allantoic membrane of chick embryos the characteristic qualities of a particular strain are preserved apparently indefinitely.

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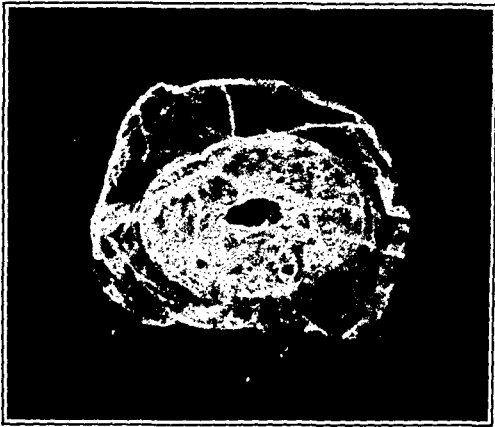
## DESCRIPTION OF PLATES

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### PLATE 99

- FIG. 1. Vaccinal lesion in the chorio-allantoic membrane; 60 hours neurotesticular virus; 50th generation.
- FIG. 2. Vaccinal lesion in the chorio-allantoic membrane; 60 hours dermal virus; 50th generation.
- FIG. 3. Section from membranal lesion; 48 hours neurotesticular virus; 50th generation.  $\times 110$ .
- FIG. 4. Section from membranal lesion; 48 hours dermal virus; 50th generation.  $\times 110$ .
- FIG. 5. Guarnieri bodies in ectodermal epithelium; neurotesticular virus; 42nd generation.  $\times 1800$ .
- FIG. 6. Guarnieri bodies in ectodermal epithelium; dermal virus; 48th generation.  $\times 1800$ .

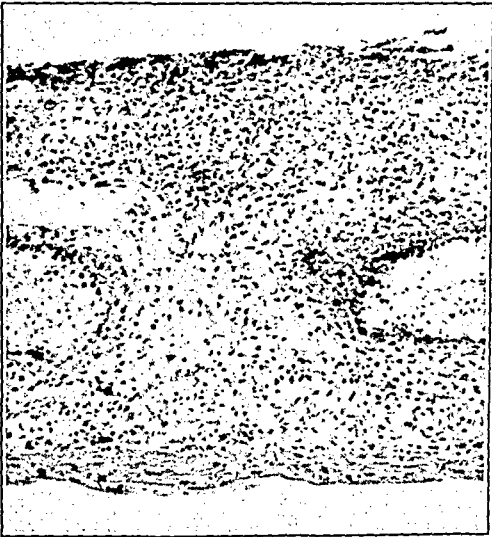




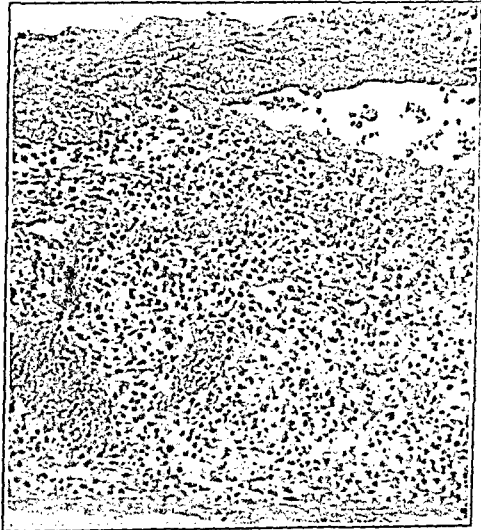
1



2



3



4



5



6

Budding

Behavior of Virus in Chorio-Allantoic Membrane

PLATE 100

FIG. 7. Membranal lesion, neurotesticular virus, showing margination of thrombocytes along blood vessel wall.  $\times 250$ .

FIG. 8. Membranal lesion, neurotesticular virus, showing area of free hemorrhage.  $\times 500$ .

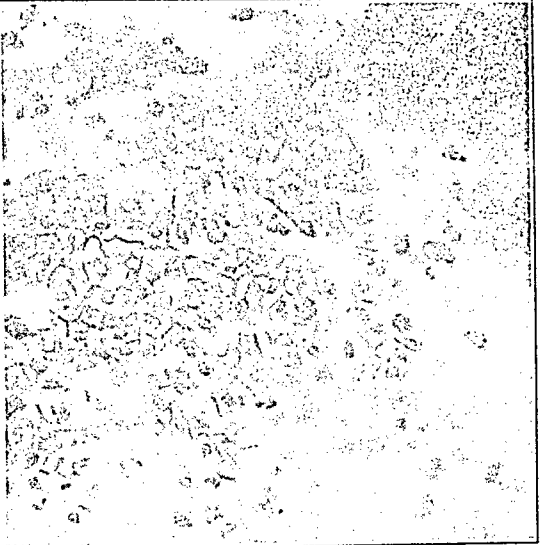
FIGS. 9 and 10. Guarnieri bodies in endothelial cells of the blood vessels. Neurotesticular virus.  $\times 1800$ .

FIG. 11. Guarnieri bodies in endodermal epithelium. Neurotesticular virus.  $\times 1800$ .

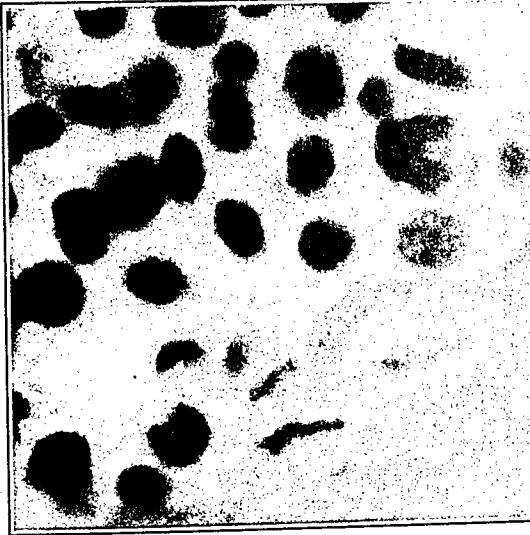
FIG. 12. Guarnieri bodies in fibroblasts of the mesodermal layer. Neurotesticular virus.  $\times 1800$ .



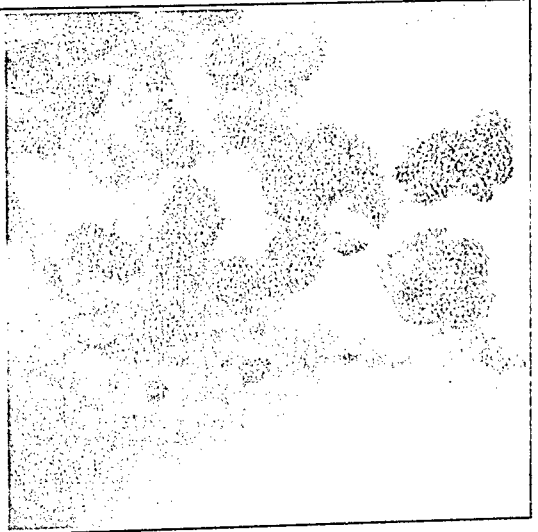
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8



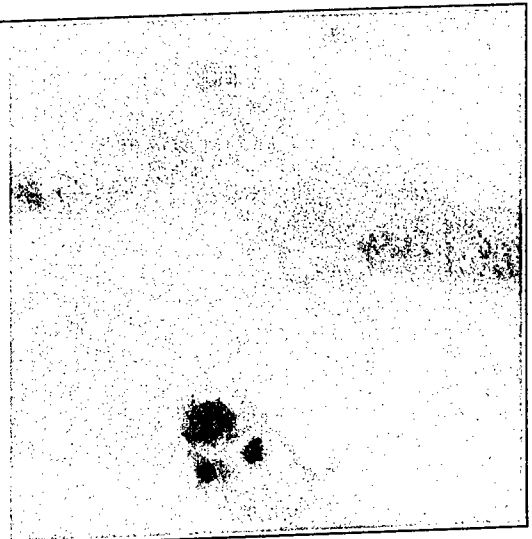
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10



11



12

Budding

Behavior of Virus in Chorio-Allantoic Membrane



# A COMPARISON OF THE GROWTH CURVES OF MALIGNANT AND NORMAL (EMBRYONIC AND POSTEMBRYONIC) TISSUES OF THE RAT \*

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The histological and biochemical similarities between embryonic and tumor tissues have led some investigators to compare mathematically embryonic and tumor growth. Bashford,<sup>1</sup> working with mice, showed that the growth rate of the embryo is approximately equal to or higher than the growth rates of 53 different types of transmissible tumors. Sugiura and Benedict<sup>2</sup> state that the growth of the Flexner-Jobling carcinoma and the fetal growth of the rat can be represented graphically, with the weight as ordinates and time as abscissas, by almost identical parabolic curves. Carrel and Ebeling,<sup>3</sup> and Fischer, Laser and Meyer,<sup>4</sup> using tissue culture methods, could not observe any differences in the growth curves or the growth rates of tumor and embryonic cells.

The recently developed method<sup>5</sup> for the graphical representation of tumor growth affords another approach to the comparative study of embryonic and tumor growth. A study was therefore undertaken to determine whether or not the previous findings could be substantiated and extended by the new graphical method. The present paper compares the growth of 3 rat tumors (Walker tumor 256, Flexner-Jobling carcinoma, and R39 sarcoma) with the embryonic and postembryonic growth of the rat.

## GROWTH OF RAT TUMORS

The mean diameter has been defined<sup>5</sup> as the cube root of the product of the three dimensions of a tumor. Growth curves of the Walker and the Flexner-Jobling rat tumors with the mean diameter as ordinates and time as abscissas have been shown to be linear. The slope of the linear growth curve represents the growth rate of the tumor and the intercept of the curve on the abscissa represents the latent period of the tumor.

\* Aided by a grant from the International Cancer Research Foundation.  
Received for publication January 20, 1936.

The average growth rate of 254 Walker tumors No. 256 was found to be 1.215 mm. per day, while the average growth rate of 95 Flexner-Jobling carcinomas was 0.649 mm. per day. Recent work <sup>6</sup> on the R39 sarcoma has shown that the average growth rate of 184 tumors was 1.777 mm. per day.

In order to compare these data on tumor growth expressed in units of mean diameter with those obtained by others on the embryonic and postembryonic growth of rats expressed in units of weight, it is necessary to use the cube root of the weight instead of the mean diameter as a measure of the size of the tumor. It was previously shown <sup>5</sup> that the weight of a tumor can be calculated by means of the formula

$$W = S (0.5236 d^3)$$

where  $W$  = the weight of the tumor in grams,  $S$  = the specific gravity, and  $d$  = the mean diameter in centimeters. Therefore

$$\sqrt[3]{W} = \sqrt[3]{S} \times \sqrt[3]{0.5236} \times d$$

This formula enables one to find the cube root of the weight of a tumor when the mean diameter and the specific gravity of the tumor are known.

It is also necessary to use a growth constant to correspond with the new measure of the size of tumors. This constant can be determined by the formula

$$k \sqrt[3]{W} = \sqrt[3]{S} \times \sqrt[3]{0.5236} \times k_d$$

where  $k_d$  = the growth constant expressed in centimeters per day and  $k \sqrt[3]{W}$  = the growth constant expressed in grams <sup>1/3</sup> per day.

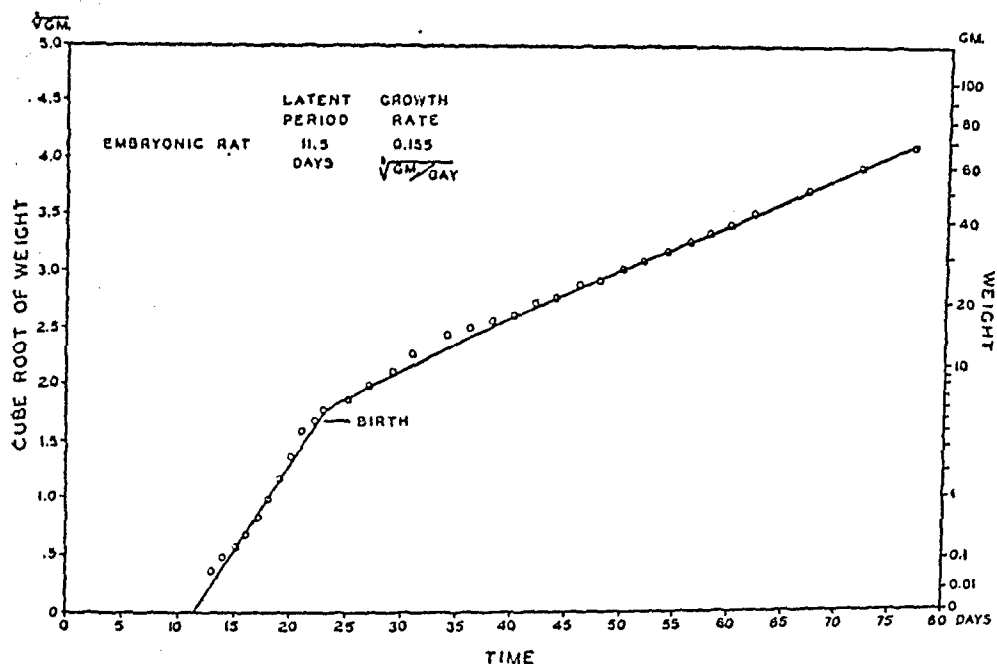
The specific gravity of the tumors has been found to be 1.038 for the Walker tumor, <sup>5</sup> 1.036 for the R39 sarcoma, <sup>6</sup> and 1.044 for the Flexner-Jobling carcinoma. <sup>7</sup> With these data on the specific gravity and the average growth rate ( $k_d$ ), it was found that the new growth constants ( $k \sqrt[3]{W}$ ) are 0.151 grams <sup>1/3</sup> per day for the R39 sarcoma, 0.099 for the Walker tumor, and 0.053 for the Flexner-Jobling carcinoma.

#### EMBRYONIC AND POSTEMBRYONIC GROWTH OF THE RAT

Stotsenburg's data <sup>8</sup> on the embryonic growth of the rat and Donaldson's data <sup>9</sup> on the postembryonic growth were used to con-

construct the growth curve in Text-Fig. 1 with the cube root of the weight as ordinates and time as abscissas.

The text-figure shows that the embryonic growth curve, like tumor growth curves, is linear. Schmalhausen<sup>10</sup> also observed a linear relation between the cube root of the weight of the rat embryo and time. The embryo has a latent period of 11.5 days and a growth rate of 0.155 grams<sup>1/3</sup> per day. This embryonic growth rate is approximately equal to the average growth rate of R39



TEXT-FIG. 1. Growth curve representing the embryonic and postembryonic growth of the rat.

sarcoma (0.151 grams<sup>1/3</sup> per day) and is somewhat higher than the average growth rates of the Walker tumor (0.099) and the Flexner-Jobling carcinoma (0.053). It seems then that the growth rates of rat embryonic and the rat malignant tissues are in the same order of magnitude.

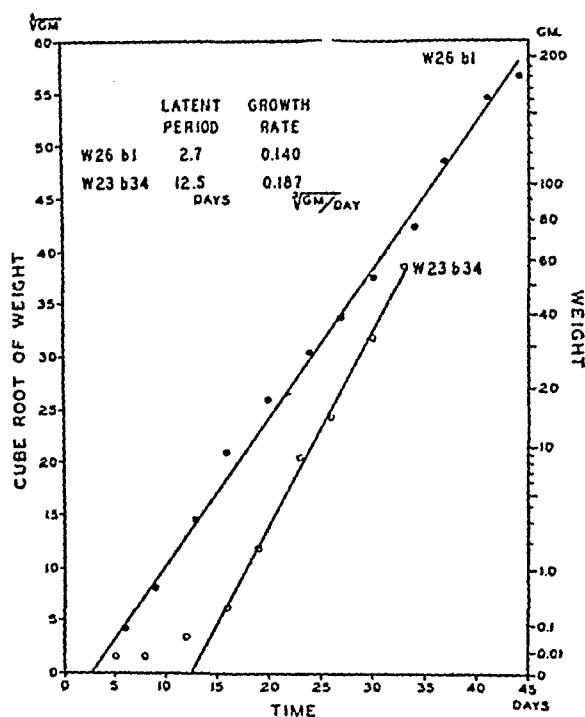
The text-figure also shows that the rat grows at a constant rate for about 11 days, attaining a weight of 5.5 gm., and then the growth rate begins to decrease. This inhibition to growth is presumably an expression of the increasing differentiation of the tissues of the rat. No inhibition, on the contrary, can be observed in the growth curves of the Walker tumor (Text-Fig. 2).

Tumor W26b1, for example, grew at a constant rate (0.140) for 40 days and was found to weigh, on postmortem examination, 175 gm.

On the other hand, the rat itself attains a weight of only 29 gm. after 40 days of growth. It follows then that the tumor has approximately the same growth rate as the embryonic rat but has a much higher growth rate than the postembryonic rat.

### DISCUSSION

The work presented shows that the growth of embryonic and malignant tissues can be represented by linear curves, and that the



TEXT-FIG. 2. Growth curves of 2 Walker tumors.

growth rates of rat embryonic and rat malignant tissues are in the same order of magnitude. From this it would appear that the malignant cell does not possess an excessive growth capacity.

Bashford<sup>1</sup> compared the growth capacities of mouse tumors and the mouse embryo. He defined the growth rate as 1000 times the reciprocal of the number of days required to develop 1 gm. of tissue. Bashford found that the mouse embryo grows faster than transplantable mouse tumors but that the growth rates of some of the tumors approximates the growth rate of the embryo mouse. Bashford concluded that the proliferative energy of tumors does not exceed that of embryonic tissue. The present investigation, using a



different species and a different method of determining growth rate, is in complete agreement with Bashford's conclusion.

The growth capacities of normal and malignant cells have also been studied in tissue culture. Carrel and Ebeling<sup>3</sup> found that the malignant fibroblasts of Crocker sarcoma No. 10 and Jensen rat sarcoma have the same growth rate as normal rat fibroblasts. Fischer, Laser and Meyer<sup>4</sup> summarize their results on Ehrlich's mouse carcinoma with the statement: "It is astonishing to find that tumor cells in culture not only do not grow more rapidly, but in many cases grow more slowly than the corresponding normal cell." These *in vitro* studies agree with Bashford's and the present *in vivo* findings that the malignant cell does not possess a growth capacity in excess of that of normal embryonic tissue.

Embryonic tissue has, in addition to its growth capacity, a tendency to differentiate. This differentiation would be expected to inhibit the growth of the normal tissue, producing a decrease in the growth rate. It should be noted that the inhibitory factor caused a marked decrease in the growth rate of the rat when the weight of the rat was 5.5 gm.

Malignant tissue like the Walker tumor exhibits no inhibition to growth, although the Walker tumor may attain a weight of 175 gm. before ulcerating or causing the death of the host. It seems that the Walker tumor has a lesser tendency to differentiate than normal tissues.

The capacity of normal tissues to differentiate has been studied *in vitro*. Fischer and Parker<sup>11</sup> succeeded in stimulating embryonic cells to differentiate in tissue culture by the use of certain media. It would be of interest to know to what extent malignant cells could differentiate under identical conditions.

This study suggests that the tumor cell in acquiring its malignancy regains its primitive growth capacity and loses more or less completely its tendency to differentiate.

### CONCLUSIONS

The growth of the Walker tumor and the rat embryo can be represented by linear curves. The growth rates of rat tumors, *in vivo* and *in vitro*, are in the same order of magnitude as the growth rate of rat embryonic tissue.

After the embryonic stage the growth rate of normal tissues is markedly diminished. This decrease in growth rate is presumably due to the differentiation of the tissues. In contrast, there is no appreciable inhibition in the growth rate of malignant tumors like the Walker tumor.

Transmissible rat tumors are characterized not by an abnormal proliferative capacity but by a lesser tendency to differentiate.

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# A BIOLOGICAL METHOD FOR STERILIZING CONTAMINATED TRANSPLANTABLE TUMORS \*

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A number of methods have been used for the sterilization of tumors accidentally contaminated in the course of transplantation. Two investigators have sterilized tumors by the use of chemicals that were stated to have a selective action on the bacterial cells — Clowes<sup>1</sup> using potassium cyanide, and Krotkina<sup>2</sup> using rivanol and trypanflavin. Other investigators were able to sterilize tumors without resorting to chemicals. Aris<sup>3</sup> succeeded in freeing mouse tumors from Spirilla by keeping the tumor suspension at a low temperature for 3 days. Rivers and Pearce<sup>4</sup> freed a rabbit carcinoma from virus III by transplanting a tumor from an animal that had been dead for more than 12 hours.

Studies<sup>5</sup> on the titration of tumor cell suspensions suggested that it might be possible to utilize the biological defence processes of the animal host for the elimination of tumor contaminants. In the previous paper it was shown that when the inoculum of a tumor cell suspension is small the tumor has a long latent period. It was stated at that time that during the prolonged latent period the natural defence mechanism of the animal host might be expected to destroy any occasional bacteria introduced with the inoculum. It was thought, therefore, that the use of a small inoculum for transplantation would aid in the maintenance of the bacteriological sterility of tumors. This report will show that heavily contaminated tumors can be sterilized by transplanting them with small inoculums.

## STERILIZATION OF 2 WALKER TUMORS

### *Experiment I*

A Walker tumor (W18a14) was transplanted, using the methods described in a previous publication.<sup>5</sup> Eighteen male rats weighing 100 gm. were inoculated, each receiving 0.1, 0.01, or 0.001 cc. of a

\* Aided by a grant from the International Cancer Research Foundation.

Received for publication January 20, 1936.

cell suspension of this tumor. At the same time an infusion broth tube and a blood agar plate were seeded with 0.1 cc. amounts of the suspension. The next day the plate had innumerable colonies, and the broth tube contained cocci and bacilli. The observations on these rats inoculated with the contaminated suspension are summarized in Text-Fig. 1.

Two of the 6 rats (Nos. 1 and 3 (Text-Fig. 1)) that received the smallest inoculums (0.001 cc.) developed tumors. Blood agar plates

C.C. OF CONTAMINATED TUMOR CELL SUSPENSION INOCULATED	NO. OF RAT	OBSERVATIONS ON TUMOR GROWTH						NO. OF COLONIES ON PLATE INOCULATED WITH 0.1 C.C. SUSPENSION	RESULTS OBTAINED ON TRANSPLANTATION		
		DAYS							C.C. OF SUSPENSION		
		9	18	23	30	37	44		0.1	0.01	0.001
0.1 (0.1 C.C. OF UNDELTED SUSPENSION)	13	●	●	●	●	●	●				
	14	●	●	●	●	●	●				
	15	●	●	●	●	●	●				
	16	●	●	●	●	●	●				
	17	●	●	●	●	●	●				
	18	●	●	●	●	●	●				
0.01 (0.1 C.C. OF SUSPENSION DILUTED 1:10 WITH SALINE)	11	●	●	●	●	●	●	INNUMERABLE	0 TAKES 3 NEGATIVES 3 DEATHS	0 TAKES 5 NEGATIVES 1 DEATH	0 TAKES 3 NEGATIVES 3 DEATHS
	7	●	●	●	●	●	●				
	8	●	●	●	●	●	●				
	12	●	●	●	●	●	●				
	9	●	●	●	●	●	●				
	10	●	●	●	●	●	●				
0.001 (0.1 C.C. OF SUSPENSION DILUTED 1:100 WITH SALINE)	1	●	●	●	●	●	●	3	4 TAKES 0 NEGATIVES 0 DEATHS	3 TAKES 0 NEGATIVES 0 DEATHS	2 TAKES 2 NEGATIVES 0 DEATHS
	3	●	●	●	●	●	●				
	2	●	●	●	●	●	●				
	4	●	●	●	●	●	●				
	5	●	●	●	●	●	●				
	6	●	●	●	●	●	●				

● TUMOR IN GOOD CONDITION  
● TUMOR WITH SUPERFICIAL NECROSIS

○ TUMOR WITH ULCERATION  
— NO. PALPABLE TUMOR

K = RAT KILLED  
D = RAT DIED

SCM

TEXT-FIG. 1. Sterilization of a contaminated Walker tumor (W18a14) by two successive transplantations with small inoculums.

inoculated with 0.1 cc. amounts of suspensions of these tumors developed only 4 and 5 colonies. This is in marked contrast to the innumerable colonies developing from 0.1 cc. of the parent-tumor suspension. The inoculation of the two slightly contaminated suspensions into other rats resulted in a high percentage of takes. Suspensions of 2 of these second generation tumors inoculated in 0.1 cc. amounts on blood agar plates and in infusion broth did not give rise to any bacterial growth during 7 days incubation. These sterile suspensions were used to carry on the tumor strain.

It has been seen that sterile tumors were obtained by two successive transplantations with small inoculums. When the inoculums

were large (0.1 and 0.01 cc.) no sterile tumors could be obtained. All the tumors developing from the 0.1 cc. inoculums either regressed or became necrotic before attaining a size suitable for transplantation. These tumors must have been heavily infected. The tumors arising from the 0.01 cc. inoculums became necrotic or ulcerated somewhat earlier than usual. One tumor (No. 11) was removed before it showed any signs of breaking down, and a suspension of the tumor was prepared. The inoculation of 0.1 cc. of the suspension on a blood agar plate gave rise to innumerable colonies of a Gram-negative bacillus. The suspension was also inoculated in varying amounts into 20 rats (Text-Fig. 1). Seven of these animals died soon after the inoculation and the rest failed to develop tumors. It would appear that the contaminating bacteria had increased in number or virulence sufficiently to cause the death of some of the animals and to prevent tumor formation.

In this experiment sterile tumors were obtained when the contaminated Walker tumor was transplanted by means of small inoculums; when large inoculums were used the developing tumors could not be transplanted because of the early necrosis and the heavy infection of the tumors.

### *Experiment 2*

Another Walker tumor (W25a8) was found on a routine transplantation to be contaminated. A blood agar plate inoculated with 0.1 cc. of a suspension of this tumor developed about 1300 colonies. The suspension contained, then, approximately 13,000 microorganisms per cubic centimeter. The contaminant on the plate was a motile Gram-negative bacillus. This bacillus produced acid and gas in glucose, levulose, dulcitol, maltose, galactose, mannitol, arabinose, xylose and rhamnose, but did not ferment lactose, sucrose, raffinose, salicin, or inulin. On Russell's double sugar the organism produced acid and gas in the butt but no acid on the slant. These reactions indicate that the organism belonged to the *Salmonella* or paratyphoid group. The pathogenicity of the organisms was tested by the intraperitoneal injection of 0.1 cc. amounts of a 24 hour broth culture in 3 rats. Two of the rats died within 6 days and the bacilli were recovered from the heart's blood. The microorganism was probably one of the paratyphoids that are endemic in rats.

The suspension contaminated with the paratyphoid bacilli was

inoculated into 18 rats (Text-Fig. 2). Five of these rats died in from 5 to 20 days. Ten of the remaining 13 rats developed tumors. Five tumors that attained a mean diameter of 18 mm. without becoming superficially necrotic or ulcerated were examined bacteriologically. The method for the determination of the degree of contamination was as follows: With aseptic precautions the tumor was removed, weighed, and ground up in a Latapie apparatus with the addition of saline equal to one-fourth of the tumor volume. The

CC. OF CONTAMINATED TUMOR CELL SUSPENSION W 25 A 8 INOCULATED	NO. OF BACILLI IN INOCULUM (30000 BACILLI PER CC. OF SUSPENSION)	NO. OF RAT	OBSERVATIONS ON TUMOR GROWTH			NO. OF BACILLI PER CC. OF TUMOR CELL SUSPENSION
			DAYS			
			10	17	24	
0.1 (0.1 CC. OF UNDILUTED SUSPENSION)	1300	36	●	●	● K	250000000 50000000
		35	●	● K		
		32	●	●	●	
		31	●	●	—	
		34	●	—	—	
		33	● D	—	—	
0.01 (0.1 CC. OF SUSPEN- SION DILUTED 1:10 WITH SALINE)	130	29	●	● K		700000 NUMEROUS
		26	—	● K		
		25	●	●	●	
		27	●	●	●	
		30	● D			
		28	● D			
0.001 (0.1 CC. OF SUSPEN- SION DILUTED 1:100 WITH SALINE)	13	19	—	●	● K	1500
		22	●	●	●	
		21	●	●	●	
		20	—	—	—	
		24	●	● D		
		23	—	● D		

● TUMOR IN GOOD CONDITION

● TUMOR WITH SUPERFICIAL NECROSIS

○ TUMOR WITH ULCERATION

— NO PALPABLE TUMOR

K- RAT KILLED

D- RAT DIED

3 CM

● TUMOR IN GOOD CONDITION ○ TUMOR WITH ULCERATION K- RAT KILLED  
 ● TUMOR WITH SUPERFICIAL NECROSIS — NO PALPABLE TUMOR D- RAT DIED

TEXT-FIG. 2. Degree of contamination and growth of tumors developing from different amounts of contaminated Walker tumor cell suspension (W25a8).

suspension was filtered through an 80-mesh Monel metal wire cloth. The filtered suspension was then successively diluted 10 times with saline from 1:10 to 1:1,000,000. Blood agar plates were inoculated with 0.1 cc. of the different dilutions and incubated for 48 hours. The colonies developing on these plates were counted and the number of organisms in 1 cc. of the undiluted suspension was estimated. This number was taken to represent the degree of contamination of the tumor.

A cell suspension of tumor No. 19 (Text-Fig. 2), arising from the smallest inoculum (0.001 cc.), was examined bacteriologically in the manner mentioned above and found to have 1500 bacilli per cubic

centimeter, whereas the parent tumor had 13,000 bacilli per cubic centimeter of suspension. Six rats were inoculated with 0.001 cc. amounts of the suspension of tumor No. 19. Four of these rats developed tumors. One of these tumors ulcerated early and another was found to be heavily contaminated with a Gram-negative bacillus. The other 2 tumors were bacteriologically sterile. It was possible, then, by two successive transplantations with small inoculums, to sterilize a Walker tumor contaminated with a pathogenic paratyphoid bacillus.

The tumors arising from larger inoculums (0.1 and 0.01 cc.) of the contaminated suspension of tumor W25a8 (Text-Fig. 2) were found to be much more heavily contaminated than the original tumor. The 2 non-ulcerated tumors (Nos. 35 and 36), developing from the 0.1 cc. inoculums, were found to have 250,000,000 and 50,000,000 organisms per cubic centimeter of suspension. One tumor (No. 29), developing from a 0.01 cc. inoculum, had 700,000 bacilli per cubic centimeter of suspension. Another tumor (No. 26), also arising from a 0.01 cc. inoculum, was found to be heavily contaminated. A blood agar plate inoculated with 0.1 cc. of a suspension of this tumor developed innumerable colonies. All these 4 tumors arising from the larger inoculums were much more heavily contaminated than the parent tumor, the suspension of which contained only 13,000 bacilli per cubic centimeter. From these results it is clear that the degree of contamination increased on transplantation when 0.1 and 0.01 cc. inoculums were used, but decreased when 0.001 cc. was used.

#### STERILIZATION OF A FLEXNER-JOBLING TUMOR CELL SUSPENSION

To confirm the findings obtained with the Walker tumor, a sterile suspension of Flexner-Jobling tumor cells was purposely contaminated with *Streptococcus viridans* so that 1 cc. of the suspension contained 1,000,000 organisms. This contaminated suspension was then inoculated into 15 rats in 0.1, 0.01, and 0.001 cc. amounts. The results of these inoculations are shown in Text-Fig. 3.

It can be seen from the text-figure that one transplantation sufficed to free the tumor from the streptococcus. This organism killed 6 of the 15 rats. One tumor (No. 1 (Text-Fig. 3)) developing from a 0.001 cc. inoculum, and 2 tumors (Nos. 8 and 6) developing

from 0.01 cc. inoculums, were sterile. It seems that the rats were able to eliminate the streptococci rapidly from the inoculation site, thus permitting the development of sterile tumors.

On the other hand, the 3 tumors arising from large inoculums (0.1 cc.) were all found to be contaminated. Suspensions of 2 of the tumors (Nos. 14 and 15) were found to have 40,000,000 and 2000 streptococci per cubic centimeter. The 3rd tumor (No. 12) ulcerated early and it was possible to cultivate *Streptococcus viridans*

CC. OF CONTAMINATED TUMOR CELL SUSPENSION INOCULATED	NO. OF STREPTOCOCCI IN INOCULUM	NO. OF RAT	OBSERVATIONS ON TUMOR GROWTH						NO. OF ORGANISMS PER CC. OF TUMOR CELL SUSPENSION
			DAYS						
			10	17	24	31	38	45	
0.1 (0.1 CC. OF UNDILUTED SUSPENSION)	100,000	14	•	•	•	•	•	K	40000000
		15	•	••	••	••	••	K	2000
		12	•	•	○	○	○	K	+
		13	D						
		11	D						
0.01 (0.1 CC. OF SUSPEN- SION DILUTED 1:10 WITH SALINE)	10000	7	•	•	•	•	K.		+
		8	•	•	•	•	•	K	0
		6	•	•	•	•	•	K	0
		10	D						
		9	D						
0.001 (0.1 CC. OF SUSPEN- SION DILUTED 1:100 WITH SALINE)	1000	1	—	—	•	•	•	K	0
		2	—	—	—	—	—	—	
		5	—	—	—	—	—	—	
		3	—	D					
		4	—	D					

● TUMOR IN GOOD CONDITION

○ TUMOR WITH ULCERATION

— NO PALPABLE TUMOR

5 CM.

K = RAT KILLED

D = RAT DIED

TEXT-FIG. 3. Sterilization of a contaminated Flexner-Jobling tumor cell suspension (F13a3) by one transplantation with small inoculums.

from a smear of the ulcer. One tumor (No. 7) arising from a 0.01 cc. inoculum started to regress. The rat was sacrificed and the tumor removed for examination. The small size of the tumor did not permit the preparation of a suspension for bacterial examination. A piece of the tumor was rubbed on a blood agar plate and placed in broth. Only 3 colonies developed on the plate and streptococci grew out in the broth in 5 days. This tumor, then, had only a small number of contaminating organisms.

In this experiment a Flexner-Jobling tumor cell suspension which was purposely contaminated with *Streptococci viridans* gave rise to



sterile tumors when 0.001 or 0.01 cc. of the suspension was used as the inoculum. When 0.1 cc. was inoculated the suspension gave rise to infected tumors.

#### EFFECT OF THE CONTAMINANT ON TUMOR GROWTH

During the course of these experiments a few observations were made on the effect of the contaminant on the gross appearance, percentage takes, growth rate and latent period of the tumors.

Certain differences in the physical characteristics of the contaminated tumors were observed. The contaminated tumors were frequently found to be quite firm. Suspensions of some contaminated tumors prepared in the usual way tended to be granular instead of turbid. Furthermore, these tumors necrosed or ulcerated very early, as can be seen in Text-Fig. 1. Cysts filled with bloody or cloudy fluid were found infrequently in the contaminated tumors. Since sterile tumors also may develop cysts, the presence of cysts in a tumor is not diagnostic of contamination. In many cases, tumors appearing perfectly normal on gross examination were found heavily contaminated.

There was some indication that the bacteria in the inoculums increased the percentage of regressions. With sterile suspensions the Walker tumor regressed in less than 2 per cent of the rats. With 0.1 cc. of the contaminated suspension W18a14 as inoculums, 3 progressive tumors and 3 regressive nodules were obtained (Text-Fig. 1). Two of the nodules that regressed appeared to be (on palpation) tumors, 1 of the nodules (No. 16) attaining a mean diameter of 15.9 mm. There is a possibility that the regressive nodules were not tumors but indurated lymph nodes. It is not possible, therefore, to state definitely that the contaminant caused the regression of Walker tumors.

It was seen above that the inoculation of 0.1 cc. of the contaminated suspension W18a14 in 6 rats gave rise to only 3 instead of the expected 5 or 6 progressive tumors. It appears, then, that the contaminant decreased the number of progressive tumors by destroying the tumor cells either before or shortly after the onset of tumor growth.

A more detailed comparison of the percentages of progressively growing tumors resulting from the inoculation of contaminated and

sterile suspensions is given in Table I. The data on the growth of the sterile tumors were taken from a previous publication.<sup>5</sup>

As can be seen from the table, 0.1 cc. of the contaminated suspensions W18a14 and W25a8 produced only 50 and 60 per cent takes, whereas 0.1 cc. of four non-contaminated suspensions produced 96 per cent takes. Statistically these differences in the percentages of takes were found to be significant. With smaller inoculums (0.01 and 0.001 cc.) the percentage takes for the contaminated and non-contaminated suspensions do not differ significantly. It

TABLE I

*A Comparison of the Percentages of Progressive Tumors Produced by Contaminated and Sterile Walker Tumor Cell Suspensions*

Suspension	No. and percentage of rats developing progressive tumors		
	Inoculum		
	0.1 cc.	0.01 cc.	0.001 cc.
4 sterile suspensions . . . . .	$\frac{27^*}{28} = 96\%$	$\frac{21}{39} = 54\%$	$\frac{12}{35} = 34\%$
Contaminated suspension . . . . (W18a14)	$\frac{3}{6} = 50\%$	$\frac{4}{6} = 67\%$	$\frac{2}{6} = 33\%$
Contaminated suspension . . . . (W25a8)	$\frac{3}{6-1^{**}} = 60\%$	$\frac{4}{6-2} = 100\%$	$\frac{3}{6-2} = 75\%$

\* Numerator = No. of rats developing progressive tumors.  
Denominator = No. of rats inoculated.

\*\* Minuend = No. of rats inoculated.  
Subtrahend = No. of rats dying presumably of infection.

appears that the small number of bacteria introduced with the small inoculums were insufficient to affect the percentage of takes.

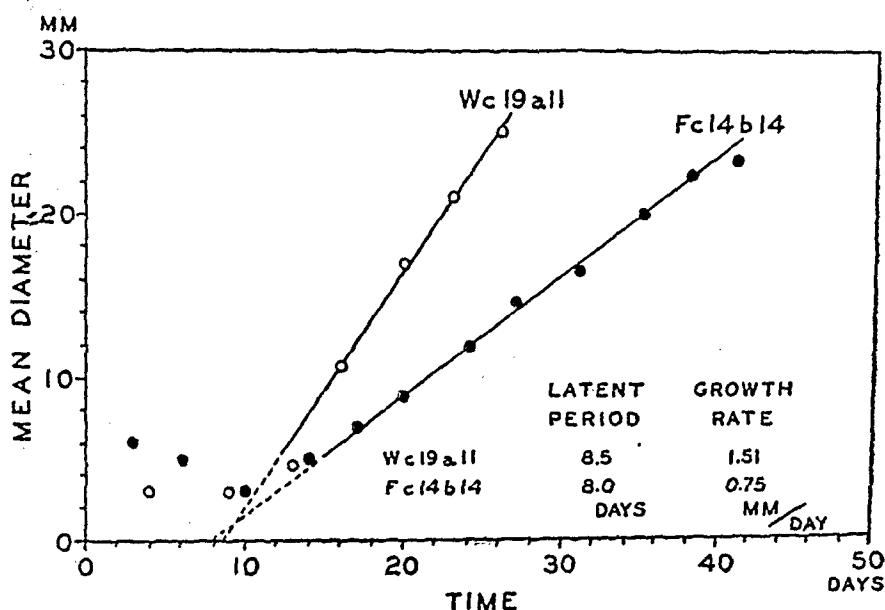
The effect of contaminants on the latent period and growth rate of tumors can be ascertained by a comparison of the growth curves of contaminated and sterile tumors. Text-Fig. 4 represents the growth curves of 2 contaminated tumors: one, Walker tumor Wc19a11, was heavily contaminated with Gram-negative bacilli (No. 11 (Text-Fig. 1)); and the other, Flexner-Jobling tumor Fc14b14, had 40,000,000 streptococci per cubic centimeter of suspension (No. 14 (Text-Fig. 3)).

The two curves in Text-Fig. 4 are linear. The slopes of these

linear curves are 1.51 mm. per day for the Walker tumor, and 0.75 mm. per day for the Flexner-Jobling tumor. These rates of growth do not differ significantly from the normal average rates of 1.215 mm. per day ( $\pm 0.311$  standard deviation) for Walker tumors, and 0.649 mm. per day ( $\pm 0.133$  standard deviation) for Flexner-Jobling tumors, as reported in a previous paper.<sup>5</sup>

A complete summary of the average growth rates of contaminated and sterile tumors is given in Table II.

The table shows that there were no marked differences in the average growth rates of contaminated and sterile tumors. It may be



TEXT-FIG. 4. Growth curves of 2 contaminated tumors (Walker Wc19a11 and Flexner-Jobling Fc14b14).

concluded that tumors harboring large numbers of bacteria can grow at a normal rate.

The table also shows that the average latent periods of tumors arising from injections of 0.1 cc. of the contaminated Walker and Flexner-Jobling tumor cell suspensions were consistently longer (9.3, 5.5 and 7.5 days) than the latent periods (1.4 and 2.7 days) of the sterile tumors. Statistical analysis of the data showed that this prolongation of the latent period is significant. During the prolonged latent periods of the tumors, nodules were present at the site of inoculation (see Text-Fig. 4). These nodules consisted presumably of the reaction tissue of the rat to the bacteria. It appears that either the bacteria or the tissue response of the animal to the bac-

teria inhibited the onset of tumor growth and prolonged the latent period.

When 0.01 and 0.001 cc. inoculums were used the latent periods of the contaminated and sterile tumors were approximately the

TABLE II

*A Comparison of the Average Latent Periods and the Average Growth Rates of Tumors Resulting from the Inoculation of Contaminated and Sterile Tumor Cell Suspensions*

Suspension	Inoculum						No. of tumors	Average growth rate
	0.1 cc.		0.01 cc.		0.001 cc.			
	No. of tumors	Average latent period	No. of tumors	Average latent period	No. of tumors	Average latent period		
<i>Walker tumor</i> 4 sterile suspensions .....	27	days 1.4	20	days 6.3	11	days 12.0	56	mm/ days 1.24
Contaminated suspension ... (W18a14)	3	9.3	4	10.9	2	16.3	9	1.23
Contaminated suspension ... (W25a8)	3	5.5	4	8.1	3	9.5	10	1.51
<i>Flexner tumor</i> 2 sterile suspensions .....	9	2.7	11	6.9	2	15.0	22	0.69
Contaminated suspension ... (F13a3)	2	7.5	3	9.0	1	13.5	2*	0.74

\* Only the contaminated tumors in the series were used to determine the average growth rate.

same. The number of bacteria introduced in the small inoculums was not sufficient to affect the latent period.

There seems to be a correlation between the latent period and the percentage takes. The bacteria in the largest inoculums used (0.1 cc.) caused both an increase in the latent period and a decrease in the percentage takes, while the bacteria in the small inoculums (0.01 and 0.001 cc.) did not affect either the latent period or the percentage takes.

It is not possible to make any generalizations from these studies

as it is obvious that the effect depends on the number and character of the contaminating organism. With the contaminants studied in this work it was observed that the bacteria in the 0.1 cc. inoculums caused, directly or indirectly, an initial inhibition of tumor growth. This inhibition was evidenced by the decreased number of progressive tumors and by the prolongation of the latent period. When this inhibition was overcome, the tumors developed at normal rates even though they harbored large numbers of organisms.

### DISCUSSION

Any method for the sterilization of a contaminated tumor by transplantation requires the destruction of the bacterial cells and the survival of the tumor cells. Various chemical and physical agents (potassium cyanide, rivanol, and low temperature) have been suggested for this purpose. Theoretical considerations indicate that it might be preferable to utilize the natural immunity of the animal to destroy selectively the bacteria inoculated with the tumor cells.

The activity of the defence mechanism of the host against a contaminated tumor inoculum depends on two factors: (1) the character and the amount of the inoculum, and (2) the resistance of the host.

The contaminated inoculum should be in the form of a tumor cell suspension. A suspension does not hinder the humoral and cellular antibacterial processes of the host, whereas a fragment of tumor tissue affords a nidus for the contaminating organisms. The amount of suspension inoculated should be the minimal amount necessary for the production of a tumor. The smaller the inoculum is, the smaller is the number of bacteria introduced into the animals. Furthermore, the smaller the inoculum, the longer is the latent period of the tumor, and the longer is the period during which the defence processes of the animal can free the site of inoculation from bacteria.

The animals used for the inoculations should be healthy and well nourished adult rats, as the resistance of these animals is likely to be high. The defence processes of the animals to be inoculated with the tumor might be strengthened, if time allows, by first immunizing with the organisms occurring in the tumor. It was not found necessary, however, to resort to this procedure with the bacteria used in this work.

The success of this method depends primarily on the capacity of the animals inoculated with the contaminated suspension to free the site of inoculation from the contaminating organism before the tumor starts to develop. There should be no difficulty in applying the method in the case of saprophytic and certain pathogenic organisms (such as *Streptococcus viridans*). The method might not be satisfactory in the case of large numbers of highly virulent organisms, especially if the contaminant is naturally infectious for the animals (such as the paratyphoid bacillus). In these experiments it was observed that only one transplantation was needed to sterilize a suspension containing 1,000,000 *Streptococcus viridans* per cubic centimeter, whereas two transplantations were required to sterilize a suspension containing only 13,000 paratyphoid bacilli per cubic centimeter. When the contaminant is naturally infectious for the animals it would be an advantage to inoculate large numbers of animals with the contaminated tumor cell suspension in the hope that one or two are naturally immune to the organism.

It may be of interest to consider the part played by the biological defence processes in the sterilization of tumors by the chemical and physical means used by other investigators. Presumably, these chemical and physical agents had, in addition to their action on the bacteria, some deleterious effect on the tumor cells. The reduction in the number of living tumor cells inoculated would be expected to prolong the latent period and thus permit the action of the defence processes of the host. In fact, Rivers reports a prolonged latent period following the use of his sterilization method. It is probable, therefore, that the biological defence mechanism is also a factor in the sterilization of tumors by chemical and physical means.

In the work on the Flexner-Jobling tumors it was noticed that the organisms recovered from the tumors were somewhat different from the original *Streptococcus viridans* that had been added to the suspension. This suggests that the tumor offers a good opportunity to induce dissociation in bacteria. The tumor acts as a nidus of dead and living tissue in which the bacteria survive a long time. The organisms in the growing tumor are probably partially protected from the immune processes of the animal host. Rivers and Pearce<sup>4</sup> have shown that viruses persist in tumors even though the animal host is immune. Under these conditions the dissociation of bacteria may be expected to be accelerated.

## SUMMARY

The routine transplantation method used in this laboratory sufficed to sterilize a Flexner-Jobling tumor cell suspension purposely contaminated with *Streptococcus viridans*, and 2 contaminated Walker tumors. Sterile tumors were obtained by transplanting once or twice with minimal amounts (0.001 cc.) of the contaminated suspensions.

The bacteria in the contaminated tumor cell suspensions caused a decrease in the percentage of takes of the tumor and prolonged the latent period, but had little if any effect on the growth rate of the resulting tumors.

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## A SIMPLE METHOD FOR THE SILVER IMPREGNATION OF RETICULUM \*

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Silver staining methods, long adopted as a standard procedure by neuropathologists, are not yet in common use for purposes of routine tissue diagnosis. Four general objections have been raised to the use of silver as a staining agent: (1) the uncertain results, (2) the need of special fixatives, (3) the use of frozen section technic, and (4) unfamiliarity with the pictures obtained. Kubie and Davidson<sup>1</sup> and Foot<sup>2</sup> have provided exact formulas to replace the empirical solutions devised by Bielschowsky,<sup>3</sup> Ramón y Cajal,<sup>4</sup> and Río-Hortega.<sup>5</sup> The equimolar solutions advocated by Kubie and Davidson<sup>1</sup> provide the chemical basis needed to ensure uniform results. With a little experience and attention to detail, silver stains can be made to yield results as constant as do the acid and basic dyes in common use. Foot,<sup>6</sup> and Foot and Foot<sup>7</sup> are largely, though not solely, responsible for making formalin-fixed and paraffin embedded tissues available for silver impregnation. The fourth objection is the least valid. The very specificity of the argyrophil reaction is one of the strongest arguments for its adoption in the field of "general" pathology. The silver methods provide an excellent supplement to the dye stains. To Masson<sup>8, 9, 10</sup> belongs much of the credit for stimulating interest in the wider application of the silver impregnation methods and showing their value in research and diagnosis.

Silver impregnation lends itself especially well to the demonstration of the reticular connective tissues. Perdrau,<sup>11</sup> Foot and Mènard,<sup>12</sup> and Laidlaw<sup>13, 14</sup> have devised useful methods for impregnating connective tissue with silver. Perdrau's formula yields almost uniformly constant results. While his method can be recommended as a standard of comparison for the evaluation of new methods, it is too tedious and time-consuming to lend itself to routine application. The method devised by Foot and Mènard<sup>12</sup> is rapid and applicable to tissues fixed either in Zenker's solution or in formalin. However, the results are not uniformly satisfactory. A series of variants re-

\* Received for publication December 20, 1935.

cently introduced by Foot and Foot <sup>7</sup> call for confusing variations in technic and, where it is desired to demonstrate particularly the connective tissues, offer no advantages over the older methods. Laidlaw <sup>13</sup> used a chemically unbalanced and highly concentrated solution of silver diammino carbonate. This is not an easy solution to work with. Therefore, there seems place for the following method which has been found to yield results as certain and constant as the Perdrau technic.

### METHOD

*Fixation:* Either 10 per cent aqueous formalin solution (4 per cent formaldehyde) or Bouin's fixative may be used. The formalin may be neutral or not. Tissues should be well fixed. They may be embedded in paraffin or in celloidin or cut on the freezing microtome. Celloidin sections may be used as such. Frozen sections should be affixed to slides or coverslips by means of a thin coating of celloidin (Wright's technic). Paraffin sections are transferred to slides or coverslips previously cleaned with strong nitric acid and with alcohol. Mayer's egg albumin medium does not effectively prevent detachment of the sections in the strongly alkaline silver impregnation solution. Three alternative methods are available, instead, for the protection of the sections during impregnation:

(1) The paraffin sections are floated on clean slides or coverslips and dried in the incubator. The slides are transferred, in turn, to xylol, absolute alcohol, and to a mixture of absolute alcohol and ether (equal parts). They are then immersed in thin celloidin, drained and partially dried in air. The celloidin film is hardened in 70 per cent alcohol and the sections placed in water (Wright's technic).

(2) The paraffin sections are floated on to a warm 1 per cent aqueous solution of gelatin, transferred to slides or coverslips and allowed to drain. The gelatin is then hardened by exposure to formaldehyde vapor. This is a modification of the Masson gelatin glue method <sup>15</sup> and is especially recommended for sections of the central nervous system. It is also useful for frozen sections where it is desired to demonstrate lipoids, using Scharlach R or Sudan III as a counterstain.

(3) Warthin's molasses and celloidin sheet principle <sup>16</sup> may be

used. This is especially advantageous when many sections are cut from a single block of tissue.

*Oxidation and Reduction:* A modification of the well known Mallory bleach is employed. The sections are oxidized for 1-5 minutes in an acidified potassium permanganate solution as follows: to 47.5 cc. of 0.5 per cent aqueous potassium permanganate solution add 2.5 cc. of 3 per cent sulphuric acid. After a brief wash in water, bleach until white in 1 per cent oxalic acid, then wash, in rapid succession, in tap water and in 2 or 3 changes of distilled water.

*Mordanting:* The sections are placed in 2.5 per cent aqueous solution of ferric ammonium sulphate (iron alum), employed as a sensitizing agent. They may be left in for a variable period (15 minutes to as long as 2 hours). The alum solution may be used repeatedly. After mordanting, the sections must be washed thoroughly in 2 or 3 changes of distilled water.

*Impregnation:* The diammino silver hydroxide solution of Kubie and Davidson<sup>1</sup> is used. To 5 cc. of 10.2 per cent aqueous silver nitrate solution add strong ammonium hydroxide solution, drop by drop, until the precipitate is just dissolved. Add 5 cc. of 3.1 per cent sodium hydroxide to the ammoniated silver solution, redissolve the resultant precipitate with a drop or two of strong ammonium solution and dilute to 50 cc. with distilled water.

The length of time of impregnation may be gauged by the time it takes the sections to become transparent. This usually occurs almost instantly. Longer impregnation causes the sections to turn a rich brown color and produces an intensely black stain after reduction of the silver. If impregnation is allowed to take place for the optimum period, only a brief wash in distilled water is necessary previous to reduction. If the time of impregnation is prolonged, the sections should be washed more deliberately.

*Reduction:* An aqueous 10 per cent formalin (4 per cent formaldehyde) is used for this purpose. The sections should be moved to and fro in this solution and reduction is complete almost immediately. After reduction the sections may be washed in tap water.

*Toning:* Gold toning is merely a refinement and may be omitted. The sections are toned in 0.2 per cent aqueous gold chloride solution (Merck's yellow gold chloride) until they turn a purplish color. The gold solution may be used repeatedly if protected from the "hypo"

fixing solution. After a brief wash the sections are fixed in 5 per cent aqueous sodium thiosulphate ("hypo") and then washed in 2 or more changes of tap water.

*Dehydration and Clearing:* Sections attached to slides with celloidin are dehydrated in 95 per cent and absolute alcohol. The celloidin is then dissolved in a solution of absolute alcohol and ether, in equal parts. This latter step is not absolutely necessary and is attended by the risk of detachment of the sections from the slides, but it is recommended because surface precipitates are thereby removed, leaving a more sharply impregnated section. The sections are then cleared as usual in xylol and mounted in balsam. Sections fastened to slides with Masson's gelatin glue are dehydrated in 95 per cent and in absolute alcohol, cleared in xylol and mounted in balsam. Celloidin sections, whether embedded as such or secondarily converted to that form by Warthin's celloidin sheet method, are partially dehydrated in 95 per cent alcohol, transferred to carbol-xylol (xylol 2 parts, melted phenol crystals 1 part), cleared in xylol and mounted in balsam.

## RESULTS

The reticulum is sharply impregnated, appearing brownish black in untuned, and dark purple in toned preparations. Nerve fibers are also impregnated, appearing almost black, whether toned in gold or not. Elastic fibers are jet black in the toned and in the untuned sections. Cartilage and the trabeculae of decalcified bone assume a pleasing golden brown color in the untuned, a purple gray in the toned preparations. If the processes of oxidation and reduction are properly completed, the background is almost colorless, the cell nuclei and cytoplasm (other than fibers) being silver-negative.

## COMMENT

This method permits impregnation of the fibers in approximately 20 to 30 minutes. In speed alone it does not quite compare with the method recently published by Wilder.<sup>17</sup> However, it permits impregnation of the finest fibers and does not call for many precise or rapid changes of the sections from one solution to another. One of its special features is its lability. The sections may be left in the alum for several hours without spoiling; they may be washed for an

indefinite period at any stage previous to impregnation in the diammino silver hydroxide solution; and they need watching and timing only while being reduced and while being impregnated in the diammino silver solution. The action of the alum mordant is peculiar also in this respect — sections that have been overstained because of prolonged impregnation may be completely destained, after reduction in the formol, by replacing them in the alum solution. Then, after a brief wash, they may be reimpregnated in the diammino silver solution and reduced in formalin. The sections will then be found as well stained as ever. This use of the alum solution is almost a duplicate of its utilization in the Spielmeyer myelin sheath stain.<sup>18</sup> However, the alum will not destain sections once they are toned in gold and fixed in "hypo." The acidified permanganate solution ensures rapid and complete oxidation of the tissues and is an improvement upon the 0.25 per cent aqueous permanganate solution commonly employed for this purpose. Oxalic acid is used for reduction because it is relatively cheap and is quite effective.

#### SUMMARY

A method, utilizing iron alum as a mordant and, if necessary, as a destaining agent, is suggested for rapid and effective silver impregnation of reticulum of tissues fixed in formalin or in Bouin's solution. The method may be conventionally summarized as follows:

1. Fix in 10 per cent aqueous formalin or in Bouin's solution.
2. Cut frozen sections or embed blocks in paraffin or in celloidin.  
Affix frozen or paraffin sections to slides by Wright's technic or by Masson's gelatin glue method, or ensheath in celloidin by Warthin's molasses-celloidin sheet method.
3. Oxidize for 1–5 minutes in acidified permanganate solution:  
47.5 cc. of 0.5 per cent aqueous potassium permanganate  
plus 2.5 cc. of 3 per cent sulphuric acid.
4. Wash in water.
5. Bleach until white in 1 per cent oxalic acid.
6. Wash in tap water and 2 changes of distilled water.
7. Mordant for 15 to 30 minutes (or longer) in 2.5 per cent aqueous iron alum.
8. Wash in 2 or 3 changes of distilled water.
9. Impregnate for a few seconds in diammino silver hydroxide.

10. Wash briefly in distilled water.
11. Reduce in 10 per cent aqueous formalin.
12. Wash in water. (If the sections are overimpregnated repeat the process from step 7.)
13. Tone in 0.2 per cent yellow gold chloride 1-3 minutes.
14. Wash in tap water.
15. Fix in 5 per cent sodium thiosulphate 5 minutes.
16. Wash well in tap water.
17. Dehydrate in 80 per cent and in 95 per cent alcohol.
18. (a) For sections affixed by Wright's method. Complete dehydration in absolute alcohol and dissolve celloidin in equal parts of absolute alcohol and ether.
18. (b) For celloidin or celloidin sheet sections. Complete dehydration in carbol-xylol (xylol 2 parts, phenol 1 part).
19. Clear in xylol.
20. Mount in balsam.

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## DESCRIPTION OF PLATE

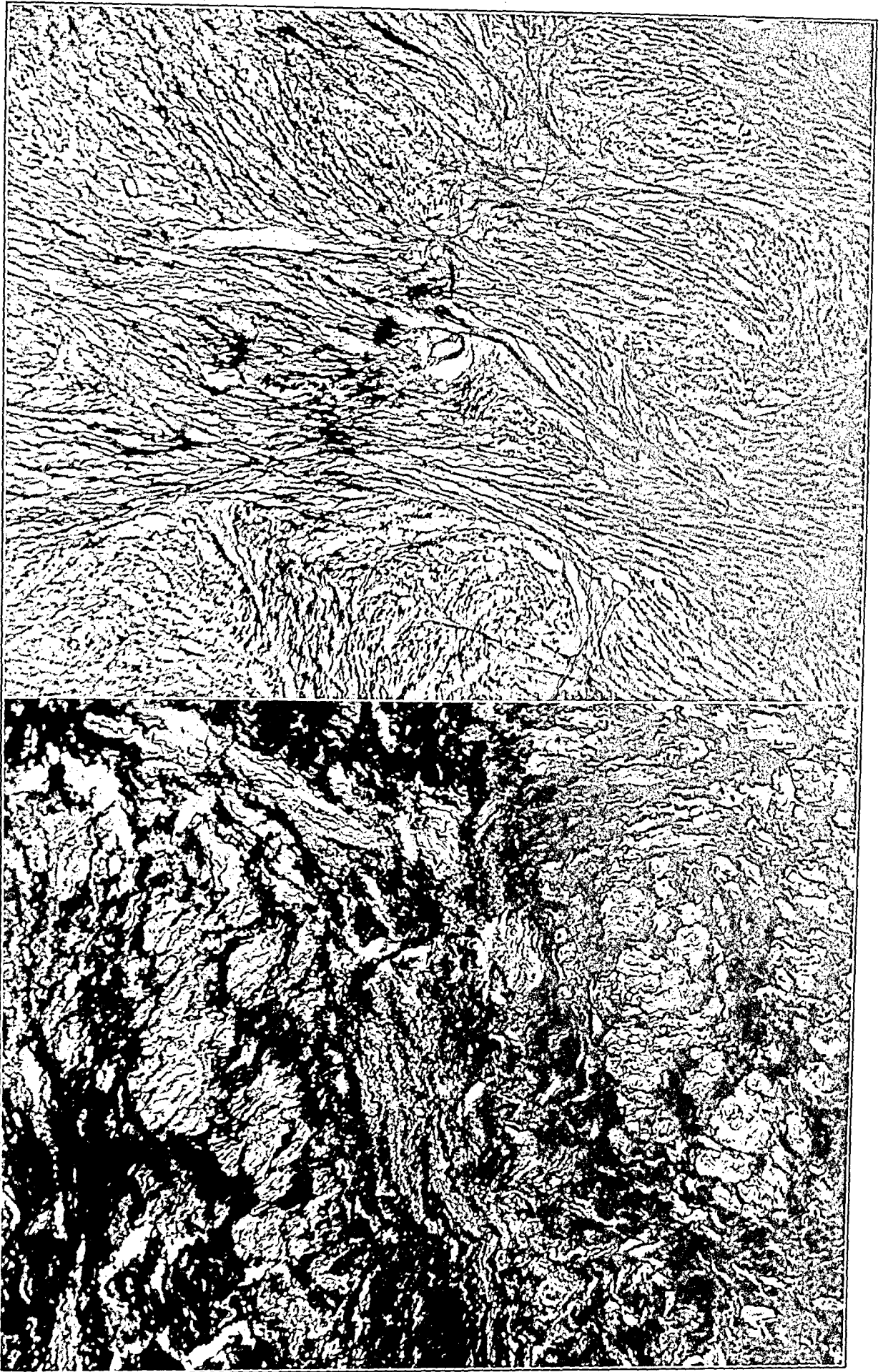
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### PLATE 101

FIG. 1. Leiomyosarcoma, showing the complex arrangement of the fibrils.  
× 105.

FIG. 2. Plexiform neurofibrosarcoma. × 170.







# SYMMETRICAL CORTICAL NECROSIS OF THE KIDNEYS \*

## REPORT OF A CASE

NEWTON EVANS, M.D., AND ELMER W. GILBERT, M.D.

*(From the Department of Pathology, College of Medical Evangelists, and the Pathology Laboratory of the Los Angeles County Hospital, Los Angeles, California)*

This peculiar lesion has excited interest for many years. The first case was reported in 1886 by Juhel-Rénoy <sup>17</sup> of France. To date 53 proved cases have been recorded, mostly by English and German observers. About 14 cases have been reported in the American literature. In 1933 Ash <sup>1</sup> made an extensive search, gathering 62 recorded cases, 18 of which were not definitely proved as some had had no portmortem examination, in others the individuals had recovered, and 1 or 2 cases had had a biopsy on one kidney only. In our search of the literature we have found 8 additional cases <sup>2, 18, 25, 27, 28, 30</sup> which we think belong to this group.

A review of the literature reveals a fairly distinct clinical picture. The patient may be male or female, but the pregnant female is by far more often the victim. The age period is wide but it is evident that the child-bearing years are the usual time of occurrence. The onset of illness is abrupt or insidious, beginning late in the last trimester of pregnancy, or even at term. In other cases symptoms make their appearance in the second trimester. The usual picture at first is that of some form of toxemia of pregnancy, *e.g.* headache, nausea, vomiting, epigastric pain, and even convulsions. Examination at this time usually reveals the absence of fetal heart tones. When the convulsions begin, spontaneous or induced delivery usually occurs shortly and a dead fetus is born. Only once were living infants born, they being twins. Almost invariably following delivery the urinary output is either markedly decreased or is nil. Such urine contains pus, red blood cells, casts and much albumin. The blood pressure is frequently elevated but about as often is normal. Nitrogen retention is common. It is remarkable that the mental state of the individual is so often clear, almost to the end, which comes generally after about 5 days of partial or complete anuria. A

\* Read before the Pathology and Bacteriology Section of the California Medical Association at the 64th annual session, Yosemite National Park, May 13-16, 1935.

Received for publication December 30, 1935.

few cases have been described in which the course appeared fairly typical and the patient recovered, but in these the urinary suppression was less complete.

The kidney lesion was notably uniform in all instances. Necrosis simulating infarction involved all the cortical portion and the columns of Bertin of both kidneys, with thrombosis of the vessels therein, but without occlusion of the arteries proximal to these vessels. The average weight of both kidneys was 413 gm. Only on three occasions did typical lesions of eclampsia appear in the liver. Many times the placenta showed signs of decomposition.

The incidence of etiological factors and symptoms from cases reviewed in the literature can be summed up as follows:

#### INCIDENCE OF ETIOLOGICAL FACTORS

<i>Sex</i>	Female .....	45 (86 %) (41 pregnant)
	Male .....	7 (14 %)
<i>Age</i>	13 to 65 yrs., average 32	

#### PRIMARY ETIOLOGICAL CONDITIONS

Pregnancy .....	41 (77 %)
Intravenous camphor, acute tonsillitis, scarlet fever, diphtheria, malaria, myocardial infarction, pulmonary tuberculosis, ruptured liver, carcinoma of prostate, cryptogenic. ....	each 1

#### INCIDENCE OF SYMPTOMS

	%
Anuria .....	100
Convulsions .....	34
Headache .....	32
Vomiting .....	55
Edema .....	55
Coma .....	42

A case presenting the classical symptoms of cortical necrosis of both kidneys has recently come under our observation and a brief report follows.

#### REPORT OF CASE

*Clinical History:* The patient, a Negress, was first seen at the age of 33 years, in March, 1926. During her 8½ months of pregnancy there were present, edema of the ankles, headache, vague abdominal pain, and vomiting. On admittance to the hospital the temperature was normal, the blood pressure 220/120, non-protein nitrogen 46 mg., and carbon dioxide combining power 40 vol. per cent. The urine contained considerable red blood cells, albumin, and a few hyaline and granular casts. Blood Wassermann was negative. A living child was

born. Ten days postpartum the blood pressure was 180/130 and 2 weeks later 190/120.

In October, 1930, after 6½ months of pregnancy, during which time nausea, vomiting, vertigo, headache, visual disturbance, and edema of the ankles were present, she was again admitted to the hospital. A convulsion had just occurred, but the patient was conscious. The blood pressure was 190/135. The urine contained 24 per cent albumin by volume, and numerous pus cells. Blood non-protein nitrogen was 33 mg. and carbon dioxide combining power 39 vol. per cent. After 4 days a dead macerated fetus was delivered. Ten days later the blood pressure was 140/100 and the urine albumin 7 per cent by volume.

On the morning of Sept. 30, 1932, she was brought to the hospital for the third and final admission. At this time she was 39 years of age, para V, gravida VIII, and 7 months pregnant. She was drowsy and unable to answer questions, but her husband stated that she had complained for several months of nausea and vomiting, epigastric pain, spells of vertigo, headache, and edema of the ankles. About September 24th she had had a convulsion, and another one on the day of admission.

Physical examination revealed moderate edema of the ankles. Fetal heart tones were absent. The urine obtained was of insufficient quantity for specific gravity determination, coagulated on heating, and contained many pus cells and fine and coarse granular casts. Red blood cells were 3,600,000 and hemoglobin 45 per cent. There was a third convulsion the evening of the day of admission and labor pains had begun. On the 2nd day a 3 pound, stillborn fetus was delivered. The placenta which followed was fragmented and decomposed. There was only a moderate amount of bleeding. The patient remained unconscious until death occurred on the 5th hospital day, 3 days postpartum.

The blood pressure on admission was 135/95. Following the convulsion that same evening it was 170/90. Preceding delivery it was 170/110; immediately following, 155/90 and 2 hours later it was 200/105. From that time until shortly before death it ranged between 190/100 and 140/80.

Blood chemistry determinations were made twice. On the 1st day non-protein nitrogen was 52, creatinine 3.5 and carbon dioxide combining power 25. The following day the non-protein nitrogen was 85, creatinine was not determined and the carbon dioxide combining power was 35.

The anuria appears to be the most significant feature. On the day of last entry an insufficient amount of urine for specific gravity determination was secured. The 2nd day none was recorded, the 3rd day only 2 ounces could be obtained and on the 4th and 5th days there was total anuria.

### POSTMORTEM EXAMINATION

Autopsy was performed 5 hours postmortem. The body was well nourished. There was moderate edema of the feet and ankles. The brain showed generalized congestion and a moderate amount of subarachnoid edema. The lungs together weighed 940 gm., were congested, edematous and presented a few subpleural petechial hemorrhages. The heart weighed 320 gm. and contained several areas of ecchymosis beneath the endocardium of the left ventricle.

The liver weighed 1600 gm., and in appearance was suggestive of fatty degeneration, but there were no lesions typical of eclampsia. The uterus weighed 730 gm. and was moderately firm. Numerous ecchymoses averaging 1 cm. in diameter were present beneath the serosa over the whole organ. The endometrium was foul smelling and was covered by soft, dark red decomposed blood clots. The left ovarian vein throughout its midportion contained an ante mortem thrombus 5 cm. in length. Both adrenals presented cortical hemorrhagic areas. The mucosa of the urinary bladder was hemorrhagic and 2-3 cc. of thick yellow fluid were present.

The kidneys together weighed 380 gm. and were about equal in size. The capsules stripped with ease and were slightly thickened and congested. The entire surface of each kidney was yellow except for mottling with small areas of hemorrhage. Several of these areas were depressed and fibrosed. On section (Fig. 1) practically the entire cortex of the kidneys had the characteristic appearance of classical infarction but for its distribution. The necrotic areas were light yellow with hemorrhagic borders. Almost all portions of the columns of Bertin were uniformly involved while the cortical infarction was interrupted at intervals by less necrotic tissue. The pyramids appeared normal except for evidence of congestion. There were a few petechial hemorrhages beneath the pelvic mucosa. The renal vessels presented no gross evidence of thrombosis.

### MICROSCOPIC EXAMINATION

Consistent with the gross pathology, the most striking histological changes were in the kidneys. The cortical structures, including all the tissue elements, were necrotic except for minute areas adjacent to the pyramids and the narrow zone beneath the capsule, which was not of sufficient depth to include any glomeruli. The greater part of the infarcted cortical tissue had a strikingly normal appearance and arrangement, except for the acute necrotic changes. The glomeruli, though somewhat swollen, looked like normal glomeruli which had suddenly necrosed. No glomeruli could be found which had been distorted or sclerosed, as would be the case in either primary or secondary contracted kidney. The sections examined showed a few sclerotic areas corresponding to the depressed surface scars seen grossly. The complete involvement of the cortical labyrinth is em-

phasized by the fact that only the rarest glomerulus could be found which took the nuclear stains as living tissue should (Fig. 2).

The convoluted tubules were all necrosed. They appeared swollen but had, for the most part, a normal arrangement and relation to each other, the necrosed epithelial cells fused with a homogeneous material filling the lumens. There were places, however, where it was evident that a minor amount of interstitial fibrosis had occurred, but it was small in amount. In numerous areas the intertubular tissue was filled with nuclear staining granular material as though a cellular exudate had collected and then disintegrated along with the necrosis of the fixed tissue elements.

The vascular elements of the cortex showed what were perhaps the most striking features. All of the vessels in the necrotic areas were likewise necrosed, but were distended with thrombotic material looking like white thrombi composed of platelets and fibrin. The most notable appearance was made by the rounded, thin walled, distorted, interlobular arteries and their branches, the afferent arterioles. For the most part these arterioles were distended with thrombotic material, but some gave the appearance of complete necrosis, the lumens closed by hyaline swelling of the walls, leaving no room for the thrombi (Fig. 3).

*Liver:* Microscopically the liver sections had a surprisingly normal appearance. In a few places the interlobular arterioles gave the faintest suggestion of hyaline degeneration.

*Spleen:* Splenic arterioles presented an intense grade of hyaline degeneration, producing an appearance in many vessels of closure of the lumens.

*Adrenal:* Section of one of the adrenals presented two striking findings — extensive focal necrosis of the cortex, and in one place a minute cortical adenoma.

## DISCUSSION

The following questions are pertinent: (1) What was the real character of the final attack? Should it be looked upon as eclampsia? Was the death an eclamptic death?

That it partook of the nature of an eclampsia is supported by its association with pregnancy and by its characteristic eclamptic symptoms. That it was not a classical non-nephritic or hepatic type of eclampsia is indicated by the absence of the characteristic hepatic

lesions and by the evident changes in the kidneys at variance with the simple tubular degenerative changes and the glomerular changes of classical eclampsia described by Bell and others. Furthermore, that it was not an eclampsia with nephritis (nephritic toxemia) is indicated by the absence of histological evidence of either an acute or chronic glomerular nephritis. Nor was it an eclampsia with chronic hypertensive disease, since the absence of cardiac hypertrophy rules out persistent arterial hypertension. On the other hand, it is reasonable to conclude that death was due to the cortical renal necrosis, anuria and uremia.

(2) What was the nature of the two previous (eclamptic) attacks? Were they non-nephritic eclampsia or were they nephritic?

That they were of the nature of eclampsia seems evident, although in the first of the two attacks, convulsions are not recorded. The absence of any examination of the patient between attacks makes it impossible to say with confidence that she did or did not have a true nephritic lesion. However, the absence of definite histological evidence of chronic nephritis at autopsy would rule out such a condition.

(3) What is the pathogenesis of the cortical necrosis? What is the cause of the vascular thrombosis?

Beginning with the first reported case, various hypotheses have been advanced as to the exact pathogenesis of the infarction. Juhel-Rénoy, in his case following scarlet fever, thought it was due to multiple emboli. Others believe it is primarily due to the action of some toxic agent on the renal tissues. Ash has recently advanced the idea of angiospasm resulting in stasis and thrombosis and consequent infarction. It is believed by almost all the writers that the thrombosis is primary and the tissue necrosis secondary, and not that the thrombosis occurs simultaneously with or subsequently to necrosis of the kidney parenchyma.

Our concept of the sequence of events in this lesion is the presence of some toxic substance in the circulating blood capable of producing injury to the capillary and arteriolar walls in large areas of the kidney cortex sufficient to cause extensive thrombosis and consequent necrosis of the tissues nourished by these vessels. It is, of course, conceivable that the changes in the involved vessel walls may include either spasm, paralysis or both.



## SUMMARY AND CONCLUSIONS

A brief review of the recorded cases of cortical necrosis of the kidneys is presented.

A case with autopsy findings, which is unique on account of previous eclamptic attacks, is reported.

The immediate cause of death was acute renal failure with anuria.

While the clinical syndrome is apparently that of eclampsia, it is not possible to assign it to one of the recognized pathological types of this disease.

A brief discussion of the possible pathogenesis of the kidney lesion is presented.

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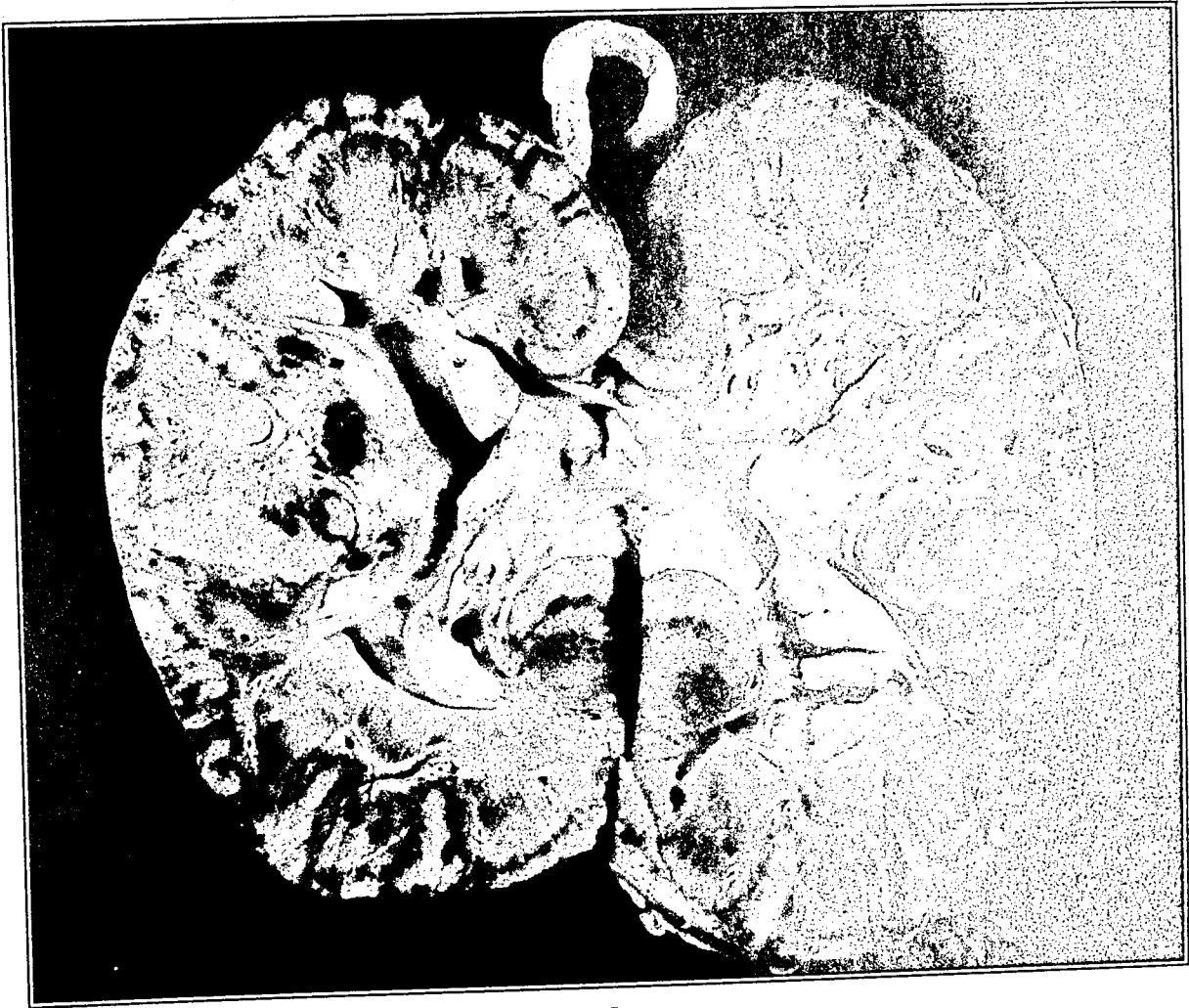


## DESCRIPTION OF PLATE

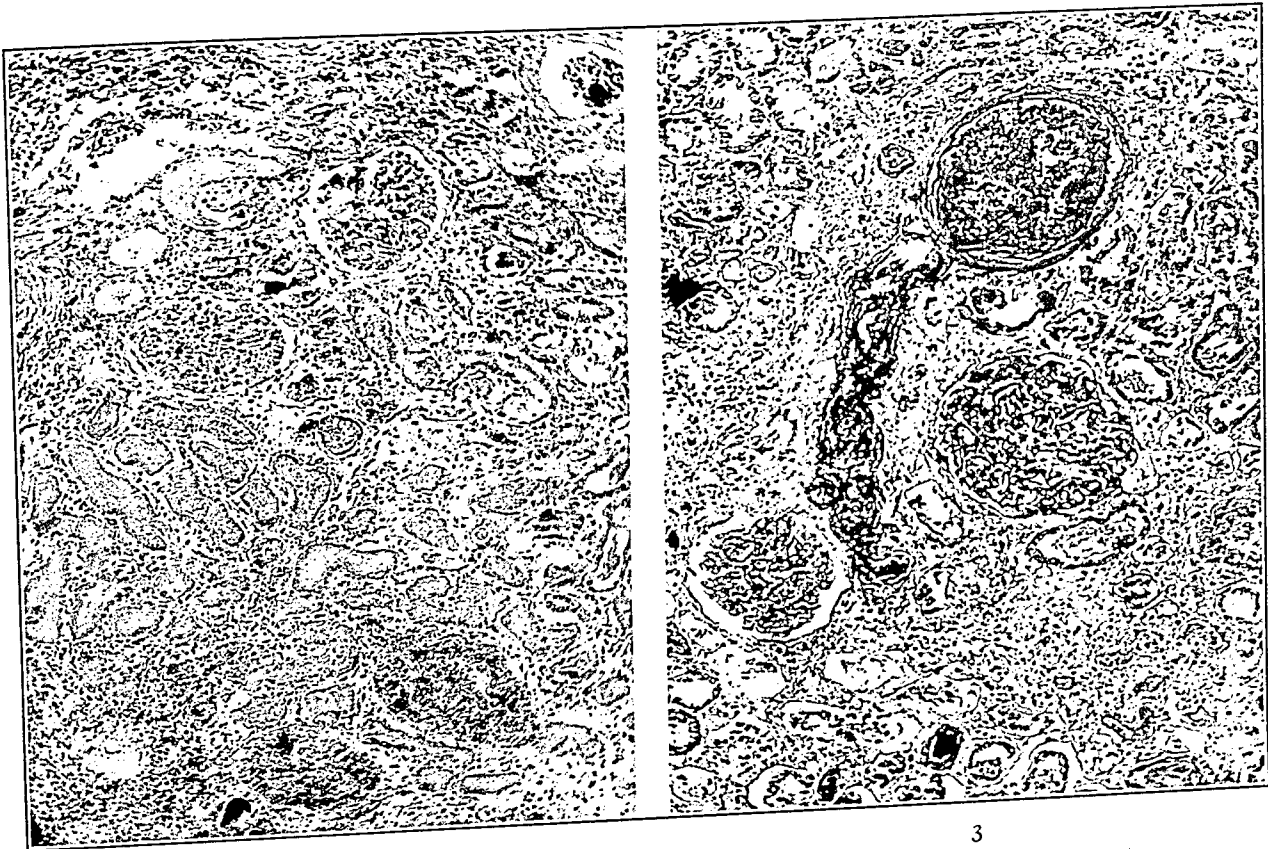
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### PLATE 102

- FIG. 1. Kidney showing practically complete necrosis of the parenchyma of cortex and columns of Bertin. The pyramids appear normal. The light colored necrotic areas have dark hemorrhagic margins.
- FIG. 2. Section of cortex of kidney showing margin of infarcted area. The upper two glomeruli and tubules are not involved in the necrotic process. Below are completely necrotic tubules and glomeruli. Hematoxylin-eosin stain.  $\times 95$ .
- FIG. 3. Section of cortex of kidney in infarcted area. Above is a dilated interlobular artery filled with thrombus. Leading from it is an afferent arteriole distended with thrombus, and its glomerulus. Mallory's aniline blue stain.  $\times 95$ .



I



2

3

Evans and Gilbert

Symmetrical Cortical Necrosis of Kidneys



# MALIGNANT TERATOMA OF THE URINARY BLADDER \*

## REPORT OF A CASE

ABOU D. POLLACK, M.D.†

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Embryonal, mixed, or teratomatous tumors of the urinary bladder are quite rare. Because of their infrequency and their interesting pathogenesis it was deemed worth while to report a case belonging to this group of tumors.

## REPORT OF CASE

*Clinical History:* The patient, a 72 year old white male, was admitted to the surgical service of the Mount Sinai Hospital Dec. 23, 1934. He complained of urinary retention of 8 days duration, requiring catheterization. For 11 years there had been increasing difficulty in urination, accompanied by frequency and nocturia. One year before admission, painless total hematuria was noted on two occasions. Sixteen years previously he had had a left ureterotomy for ureterolithiasis, following which there was complete relief from all symptoms.

*Physical Examination:* The patient was well developed and well nourished. The heart was not unusual. The systolic blood pressure was 170, the diastolic 70 mm. Hg. The lungs were clear. Both kidneys were palpable but not tender. Rectal examination disclosed a considerably enlarged prostate which was firm and homogeneous in consistence.

*Laboratory Data:* The hemoglobin was 65 per cent (Sahli). The urine contained 2-4 red blood cells, 1-3 white blood cells, and a rare granular cast per high power field. The phenolsulphonephthalein test showed 75 per cent excretion in 6 hours. The blood urea nitrogen was 20 mg. per 100 cc.

*Course of Illness:* Intravenous urography revealed a greatly dilated left kidney pelvis and calyces. The left ureter was also considerably dilated down to the level of two large calculi impacted just above the ureterovesical junction. The right side was not visualized. Through the cystoscope a pronounced intravesical and intra-urethral enlargement of the lateral lobes of the prostate was observed. Suprapubic cystotomy and bilateral vas ligation were performed Jan. 4, 1935. At the same time a left ureterolithotomy was done and two stones were removed from the ureter. Postoperatively there was no significant rise in the blood urea. A mild infection of the ureterotomy wound and a perianal abscess complicated the convalescence, but both were finally controlled. With suprapubic drainage the general condition improved and the phenolsulphonephthalein excretion rose to 90 per cent in 6 hours. On Feb. 8, 1935, a second stage suprapubic prostatectomy was performed. An enormous prostate composed of a median and two lateral lobes was removed. The pathological diagnosis was fibro-adenoma of the prostate. Following this procedure there was a steady rise in temperature, associated with the appearance of râles in the lower

\* Received for publication January 31, 1936.

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lobes of both lungs. A roentgenogram of the chest revealed bronchopneumonia. In the film an unusual area of consolidation was noted in the left lower lobe. The blood urea nitrogen rose to 50 mg. per 100 cc. There was a terminal *B. coli* bacteremia. The patient lapsed into coma and died Feb. 15, 1935.

### POSTMORTEM EXAMINATION

The body is that of an emaciated but well developed white male, 72 years of age. There is a recent, well healed incision in the left hypogastrium and an open suprapubic incision through which a rubber drainage tube passes into the bladder. On opening the bladder one finds a deep excavation in the region of the apex of the trigone, which marks the site of prostatectomy. The excavation is filled by a mass of grumous hemorrhagic material. Elsewhere the bladder is heavily trabeculated, the mucosa deeply engorged and hemorrhagic. Numerous small diverticulums are present in the posterior wall. Just medial to the left ureteral orifice a large diverticulum 4 cm. in diameter is found. It is not visible from the mucosal surface of the bladder and lies immediately anterior to the intramural portion of the left ureter, apparently obstructing it at this point. The neck of the bladder is greatly thickened throughout its entire circumference. The wall measures 3 cm. on the left side and 2 cm. on the right. The thickening diminishes gradually upward toward the fundus and downward toward the prostatic shell. Sections through the thickened portions reveal diffuse infiltration of the bladder wall by soft, white, medullary tumor tissue which is mottled by hemorrhage. The tumor is restricted to the muscular wall of the bladder; there is no bulging of the tumor into the lumen of the bladder, and it does not appear anywhere on the surface. Posteriorly the seminal vesicles immediately overlie the infiltrated wall of the bladder. The seminal vesicles themselves, however, and the vasa deferentia are discretely outlined and have no connection with the underlying tumor. The spermatic cords, testes and epididymes show no tumor.

A single metastasis is found in the lower lobe of each lung. These are 6 cm. and 3 cm. in diameter, respectively, and white, medullary and friable. The peripheral portions of these nodules are markedly hemorrhagic. No other metastases are found.

There are present also, bilateral hydronephrosis, hydro-ureter and ascending pyelonephritis. Bronchopneumonic areas are found in the



lungs. The liver is notable for a diffuse fibrosis producing a disorganization of the normal lobular architecture. Multiple congenital cysts of the head of the pancreas and a small cavernous hemangioma of the liver are also found.

### MICROSCOPIC EXAMINATION

The prostate, which had been removed surgically, was reexamined. The entire specimen, which had been fixed in Kaiserling's solution, was recut and many blocks were made. This examination again disclosed simple fibro-adenoma.

The primary tumor in the bladder is found to be a diffusely infiltrating, non-encapsulated growth whose tendency toward invasion of the blood vessels is striking. Tumor necrosis is moderate. The tumor has two distinct components — an epithelium and a stroma. A characteristic feature of the epithelium of this tumor is the frequent transition from disoriented, simple sheets of cells into glandular structures (Fig. 1). The epithelial elements are arranged in nests, sheets and simple acini. Some acini are more elaborate and form structures roughly resembling embryonal renal glomeruli (Fig. 2), such as are not infrequently seen in mixed tumors of the kidney. This characterization is not intended, however, to have more than descriptive significance. The epithelial cells are small. The nuclei approximate the lymphocyte in size, are round and fairly uniform in appearance with distinct membranes. The chromatin material is finely dispersed and distributed also as small, deeply basophilic granules just under the nuclear membrane. There are no nucleoli. Mitotic figures are frequent. The cytoplasm of the epithelial cells is ill defined, faintly acidophilic and scanty.

The second component of the tumor is designated as stroma, although it forms an integral part of the neoplasm. In fact, there are many areas in which it is difficult to distinguish between epithelial and stromal cells. The major portion of the stroma bears a considerable resemblance to embryonal mesenchyma. The cellularity varies in density, ranging from a rather loose reticular to a fairly compact tissue whose cells assume a spindle shape and a fasciculated arrangement. The cells are of about the same size as the epithelial cells. Their nuclei vary in shape from round to long spindle forms. The nuclear membrane is discrete and delicate, sometimes wrinkled.

lobes of both lungs. A roentgenogram of the chest revealed bronchopneumonia. In the film an unusual area of consolidation was noted in the left lower lobe. The blood urea nitrogen rose to 50 mg. per 100 cc. There was a terminal *B. coli* bacteremia. The patient lapsed into coma and died Feb. 15, 1935.

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### MICROSCOPIC EXAMINATION

The prostate, which had been removed surgically, was reexamined. The entire specimen, which had been fixed in Kaiserling's solution, was recut and many blocks were made. This examination again disclosed simple fibro-adenoma.

The primary tumor in the bladder is found to be a diffusely infiltrating, non-encapsulated growth whose tendency toward invasion of the blood vessels is striking. Tumor necrosis is moderate. The tumor has two distinct components — an epithelium and a stroma. A characteristic feature of the epithelium of this tumor is the frequent transition from disoriented, simple sheets of cells into glandular structures (Fig. 1). The epithelial elements are arranged in nests, sheets and simple acini. Some acini are more elaborate and form structures roughly resembling embryonal renal glomeruli (Fig. 2), such as are not infrequently seen in mixed tumors of the kidney. This characterization is not intended, however, to have more than descriptive significance. The epithelial cells are small. The nuclei approximate the lymphocyte in size, are round and fairly uniform in appearance with distinct membranes. The chromatin material is finely dispersed and distributed also as small, deeply basophilic granules just under the nuclear membrane. There are no nucleoli. Mitotic figures are frequent. The cytoplasm of the epithelial cells is ill defined, faintly acidophilic and scanty.

The second component of the tumor is designated as stroma, although it forms an integral part of the neoplasm. In fact, there are many areas in which it is difficult to distinguish between epithelial and stromal cells. The major portion of the stroma bears a considerable resemblance to embryonal mesenchyma. The cellularity varies in density, ranging from a rather loose reticular to a fairly compact tissue whose cells assume a spindle shape and a fasciculated arrangement. The cells are of about the same size as the epithelial cells. Their nuclei vary in shape from round to long spindle forms. The nuclear membrane is discrete and delicate, sometimes wrinkled.

The chromatin material assumes a very fine reticulated appearance. In some areas the nuclei are large, exceedingly irregular and hyperchromatic. Mitoses are only occasionally seen. The cytoplasm is faintly acidophilic and vague in outline. The reticular areas owe their appearance to an interlacing arrangement of delicate cytoplasmic processes. The stroma contains many dilated capillaries. Small hemorrhages are conspicuous everywhere. Finally, multiple small centers of embryonal cartilage are found within focal concentric condensations of stromal cells (Fig. 3).

The metastases in the lung exhibit the same histological structure as the primary tumor. Frequently, fragments of tumor tissue are found in small but considerably distended pulmonary arteries, adherent to the wall with beginning invasion, or free in the lumen (Fig. 4). It is possible that in addition to growth there may even have been differentiation of tumor tissue in such metastases — for example, elaboration of embryonal cartilage out of mesenchymal stroma. Large areas of infiltrated alveoli show necrosis and hemorrhage. Invasion of a small bronchus by tumor tissue is also found.

### DISCUSSION

A discussion of teratomatous tumors in general lies beyond the scope of this report. Ewing's<sup>1</sup> comprehensive treatment of the subject remains a standard. A short consideration by Rosedale<sup>2</sup> has appeared recently.

In any examination of a teratoma two questions must arise, namely origin and composition. Concerning the origin of these tumors, Ewing has grouped them into three categories: "(1) Teratomas derived from aberrant sex cells and found chiefly in the sex glands. (2) Extragenital teratomas, many of which approach the development of the parasitic fetus and which may be derived from isolated nearly totipotent blastomeres, or from early budding of the blastoderm. (3) Teratomas (or teratoids) derived from multipotent material of distinct regional stamp and reproducing the organs of these regions." These categories will, it appears, include most cases. Other concepts of genesis represent for the most part elaborations and may in some instances have validity. At any rate, it is apparent that no single pathogenetic principle may be adduced as an explanation for the origin of all teratomatous tumors.

The question of the ultimate composition of such tumors is a more difficult one. Why one tumor goes on to complete and varied tissue or even organ differentiation, while another of apparently similar origin exhibits only a very early embryonal character, remains unsolved. Borrowing from experimental embryology, Willis<sup>3</sup> considers tissue and organ elaboration in teratomas an expression of "tissue correlations." For example, young, growing, central nervous system tissue may evoke chondrification in the neighboring plastic mesenchyma. Such "tissue correlations" may, he contends, obtain in teratomatous tumors as well as in normal ontogeny.

Finally, a consideration of malignant change in teratomas must fall into the general problem of neoplastic growth.

The tumor described in this paper is designated as a teratoma in that it falls into Ewing's third category — "derived from multipotent material of distinct regional stamp and reproducing the organs of these regions." Here, however, the embryonal character is retained and no organs are evolved. It is well to refer briefly to similar tumors described heretofore.

Mixed tumors occurring in the bladder were considered by Wilms,<sup>4</sup> who cited 3 cases. One, that of Shattock, concerned a 55 year old male with a polypoid tumor in the region of the right ureteral meatus. Microscopically the tumor was found to be composed of smooth muscle cells, well formed cartilage, and round and spindle-celled sarcomatous elements. A 2nd case, that of Benecke, occurred in a 75 year old male. In this case the tumor was similar in gross appearance and in location to that reported by Shattock. Histologically this tumor was found to be comprised of a pleomorphic sarcomatous tissue containing elastic fibers, cartilage and bone. The 3rd citation of Wilms is a case reported by Livio and deals with a multiple polypoid tumor of the lower third of the bladder in a 13 year old male. This tumor was composed of embryonal striated muscle fibers. Wilms was of the opinion that such tumors are found in the urinary bladder only of males, and that the homologue of this type of tumor in the female is a polypoid tumor of comparable histology sometimes found in the vagina or cervix. This, together with the fact that the tumors in the male bladder occur either in the trigone or in the region of the ureteral meatus, led him to believe that both the cervical or vaginal mixed tumors in the female and the vesical mixed tumors in the male have a single origin. The Wolffian

duct in its caudal growth, he thought, carries down mesodermal elements of sclerotomal and myotomal potencies.

Since the time of Wilms, similar tumors have been described, some in females. Hückel<sup>5</sup> mentions a case reported by Mönckeberg, that of a pedunculated tumor occurring in the trigonal area of the bladder in a 23 year old female. Smooth and striped muscle cells were found histologically. He cites also, Hüsler's case of a 1½ year old male child with a lobulated trigonal tumor which he called "fibroma oedematosum myoenchondromatosum." The tumor also contained striated muscle fibers. The 3rd case cited by Hückel is one of Ried's — a 57 year old male with a bladder tumor whose point of origin in the bladder was no longer recognizable. Histological examination revealed bone and osteoid tissue in a sarcomatous matrix. Hückel contends with Gruber<sup>6</sup> that it is not necessary to evoke Wilms' explanation of the origin of these tumors — that it is sufficient to recall the mesodermal derivation of the trigonal area of the bladder and assume the realization of the potentialities of mesoderm in the formation of such tumors. Such a generalization is probably unjustified, however. Mixed tumors, such as those described, are not found everywhere. It is certainly more than a coincidence that they should occur where they do. The occurrence, furthermore, of such tumors in the bladder of the female is also not in discord with Wilms' theory. The Wolffian duct, it is recalled, joins the primitive indifferent cloaca of the early embryo. The cloaca later becomes differentiated into a ventral urogenital sinus and a dorsal rectum. If Wilms' thesis be correct, then the possibilities for the development of tumors such as those under consideration can exist in all tissues developing from the region of juncture of the mesonephric duct and the ventral portion of the cloaca.

Teratomatous tumors of the urinary bladder have been described also by Wright-Smith<sup>7</sup> and by Teleky.<sup>8</sup> That reported by the latter was found in a 35 year old female and consisted of a pedunculated growth in the region of the trigone. Histologically, derivatives of all three germ layers were represented. This tumor may have a different origin from those mentioned and that reported here.

Gabe<sup>9</sup> reported a case of sarcomatous carcinoma of the urinary bladder and reviewed the literature concerning sarcoma. The total number of cases of sarcoma of the bladder reported up to 1932 is 130. Six cases of mixed carcinoma and sarcoma have been reported. The re-

ported cases of sarcoma include many histological varieties — fibro-, angio-, myo-, myxo-, osteochondro-, round-celled and spindle-celled sarcoma. It is possible that some of these tumors are really primary teratomas that have become unilateral in their growth potentialities.

It is believed that the tumor described in this paper falls into the group of neoplasms that Wilms considered as having origin in mesoderm carried down to the bladder by the caudally growing Wolffian duct. Such mesoderm may exhibit the potentialities of embryonal sclerotome, myotome or nephrotome. In this tumor no striated muscle cells were found. The sarcomatous and cartilaginous elements are looked upon as being derived from the sclerotome, the epithelial elements from the nephrotome. The tumor is in many respects similar to the tumor of the kidney reported by Wilms, to which it may indeed be related.

The simultaneous occurrence of multiple tissue or organ maldevelopment has often been noted. This subject has been summarized by Gruber<sup>10</sup> in connection with congenital cysts of the pancreas. It is interesting that in addition to the teratoma of the bladder, in the case reported here, a cavernous hemangioma of the liver and multiple congenital cysts of the head of the pancreas were also found.

#### SUMMARY AND CONCLUSIONS

A malignant teratoma of the urinary bladder occurring in a 72 year old male is described. Its relation to similar tumors mentioned in the literature is discussed. It is concluded that the tumor described here falls into that group first considered by Wilms as being derived from dysontogenetic rests of the dorsal mesodermal segments carried down to the bladder anlage by the caudally growing Wolffian duct.

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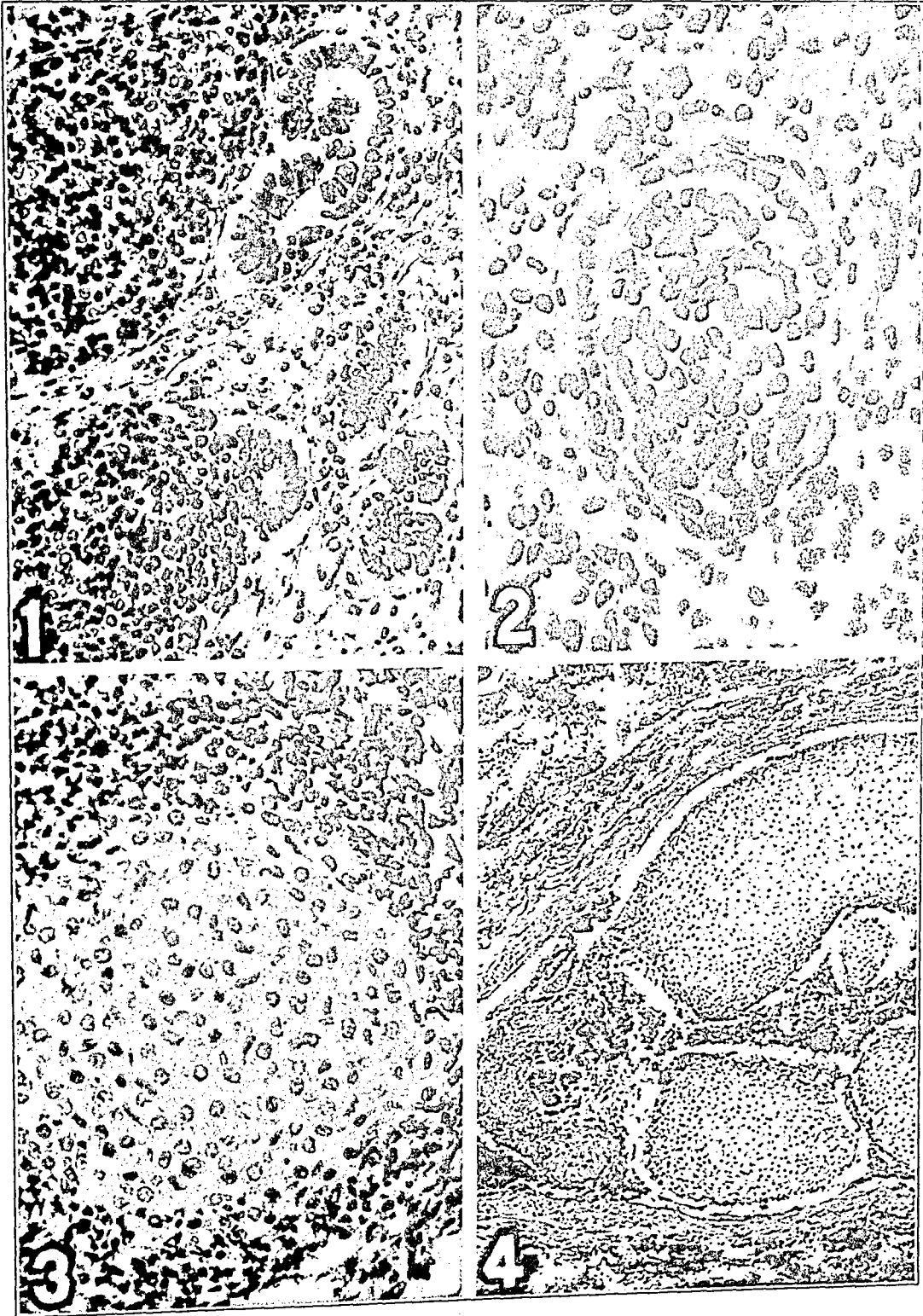
#### DESCRIPTION OF PLATE

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##### PLATE 103

- FIG. 1. Section through a typical area of the tumor showing epithelial and stromal elements.  $\times 200$ .
- FIG. 2. Section through tumor showing epithelial cells simulating an embryonal glomerulus.  $\times 400$ .
- FIG. 3. Section through primary tumor showing condensation of mesenchymal stroma to form a nest of embryonal cartilage.  $\times 220$ .
- FIG. 4. Section through metastatic nodule in lung showing tumor tissue in pulmonary artery. All elements of tumor are present — epithelium, mesenchymal stroma and embryonal cartilage.  $\times 110$ .





Malignant Teratoma of Urinary Bladder

Pollack



## A LEAD HEMATOXYLIN STAIN FOR AXIS CYLINDERS \*

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Hematoxylin and its oxidized derivative hematein have the property of combining with various metals to form colored compounds. Some of these are soluble in water or other fluid and some are not. A few are valuable as staining reagents and have long been in use in the laboratory — as for example the combinations of hematein with aluminum, iron, chromium, copper and tungsten. The staining properties of the compounds depend both on the metals and on the salts of them employed.

There has always been a need in the technical work of pathology for a reliable stain for the axis cylinder processes of ganglion cells. The silver stains give beautiful pictures when they work but are unsatisfactory to the pathologist because they are unreliable.

The following method is not perfect but is simple and can be depended on if the tissue is obtained soon after death and is properly fixed. The stain is a combination of unripened hematoxylin with lead and is insoluble in water. Therefore the staining method involves two steps — impregnation of the tissue with lead chloride followed by the application of a solution of hematoxylin. The accompanying photomicrographs give an idea of the possibilities of the method.

1. *Fix* in 10 per cent neutral formalin.

The tissue should be as fresh as possible and sections of it should not be over 3 mm. thick.

2. *Mordant* in a saturated aqueous solution of lead chloride (slightly over 1 per cent) for 6 weeks at room temperature or for 7 days in an incubator at 37° C. Change fluid at end of 24 hours and once or twice later.

3. *Wash* in running water for 24 hours to get rid of the unfixed lead chloride so as to prevent precipitation of it by alcohol.

4. *Preserve* in 80 per cent alcohol.

5. *Embed* in celloidin or paraffin. Celloidin is as a rule preferable for the spinal cord; paraffin for sympathetic nerve ganglia.

\* Received for publication April 28, 1936.

6. *Staining.* Excellent results can be obtained by using the following solution:

Powdered hematoxylin . . . . . 1 to 5 mg.

(an amount approximately the size of a pin-head to  $\frac{1}{3}$  of a match-head)

Distilled water rendered alkaline by excess of calcium carbonate in the container . . . . . 10 cc.

Dissolve the hematoxylin in 1 cc. or less of 95 per cent alcohol and add to it the alkaline water. The mixture takes on a reddish color.

The coarser nerve fibers are readily stained in  $\frac{1}{2}$  to 1 hour at room temperature. The fine sympathetic nerve fibers require up to 3 hours in the paraffin oven at about 54° C. in order to stain them intensely.

A more differential stain can be obtained in the following manner. Put the unstained sections into a weak solution of iodine for 1 minute.

Iodine . . . . . 0.1 gm.

Potassium iodide . . . . . 0.2 gm.

Distilled water . . . . . 100.0 cc.

Wash off in water and extract the iodine more or less completely in 2 or 3 changes of 95 per cent alcohol (3 to 5 minutes). Do not use sodium thiosulphate. Transfer the sections to water and stain as above directed.

7. Soak the stained sections in several changes of tap water for 1 to 2 hours to render sharper the blue color.

8. Dehydrate in alcohol, clear in oleum origani Cretici (xylol for paraffin sections) and mount in Canada balsam.

*Results:* Axis cylinders sharp blue to bluish black; nuclei blue; cytoplasm light to dark blue; neuroglia fibrils dull bluish gray; collagen, reticulum and myelin practically colorless; elastic fibers variable — colorless to fairly dark blue depending on length of staining time.

Fair results can be obtained by this staining method with sections of formaldehyde fixed tissue if they are first mordanted for an hour or more in the lead chloride solution and then washed thoroughly in several changes of tap water.

The color of the nerve fibers will be found to deepen a little on exposure to light owing probably to ripening of the hematoxylin.

but already ripened hematoxylin can not be used owing to the slow formation of a precipitate.

This method of staining axis cylinders will probably find its chief value in the study of lesions of the spinal cord and of peripheral nerves. It is unfortunate that elastic fibers are also stained but their structure and arrangement will usually prevent confusion and they can always be demonstrated in other sections by means of Weigert's resorcin fuchsin method.

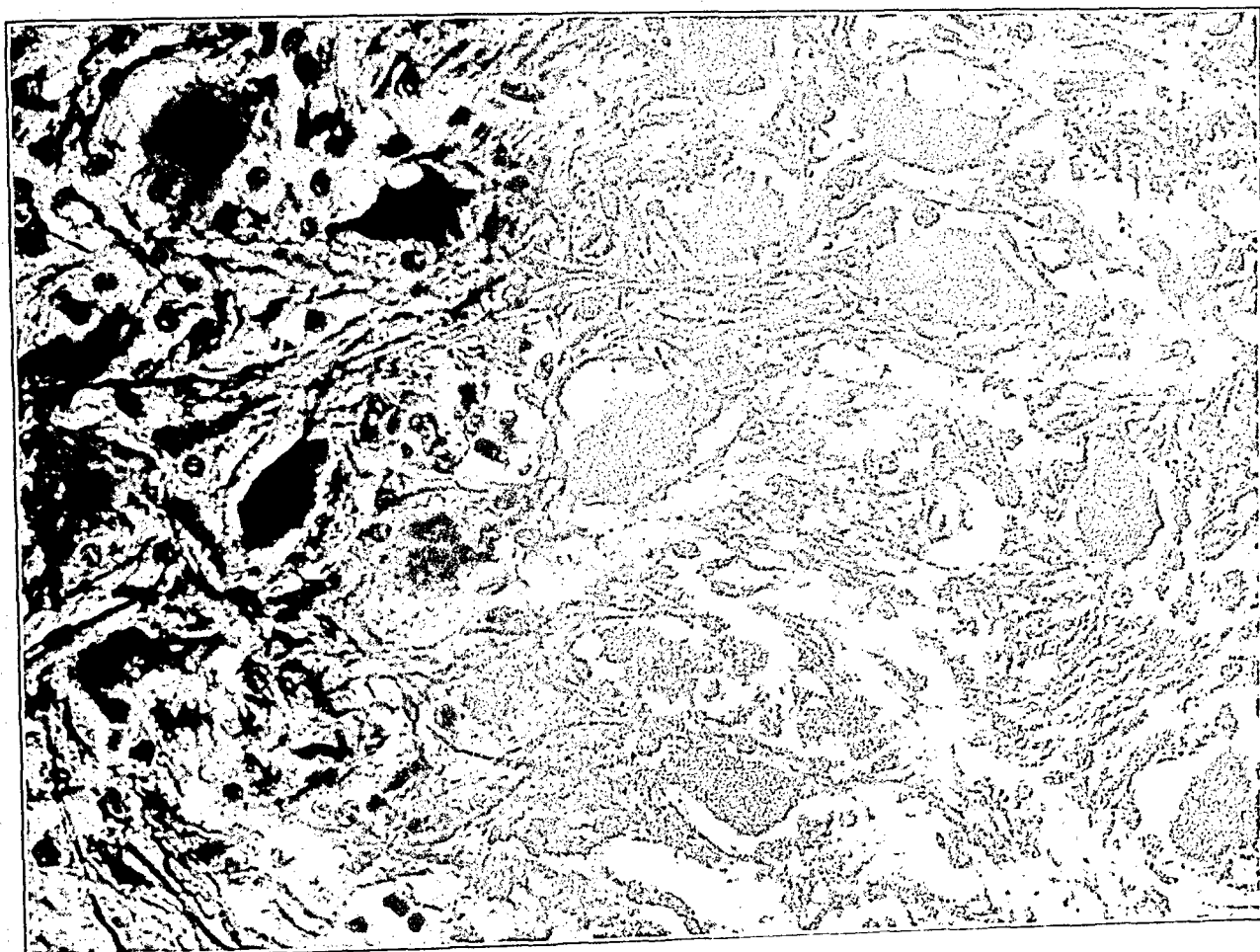
## DESCRIPTION OF PLATES

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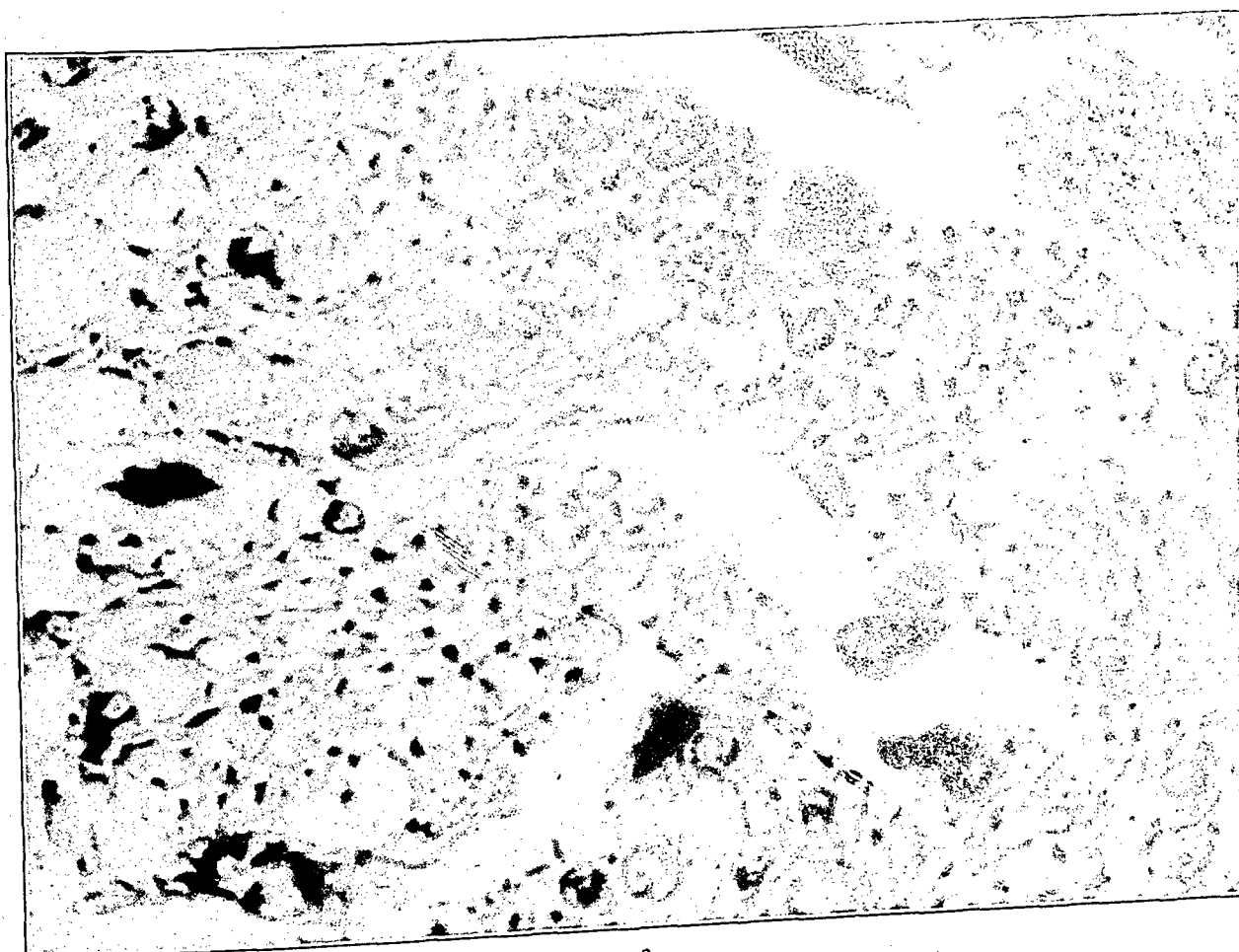
### PLATE 104

FIG. 1. Section of a sympathetic ganglion from the celiac nerve plexus showing ganglion cells, dendritic processes and sympathetic nerve fibers.  $\times 500$ .

FIG. 2. Sympathetic nerve fibers from center of ganglion.  $\times 2250$ .



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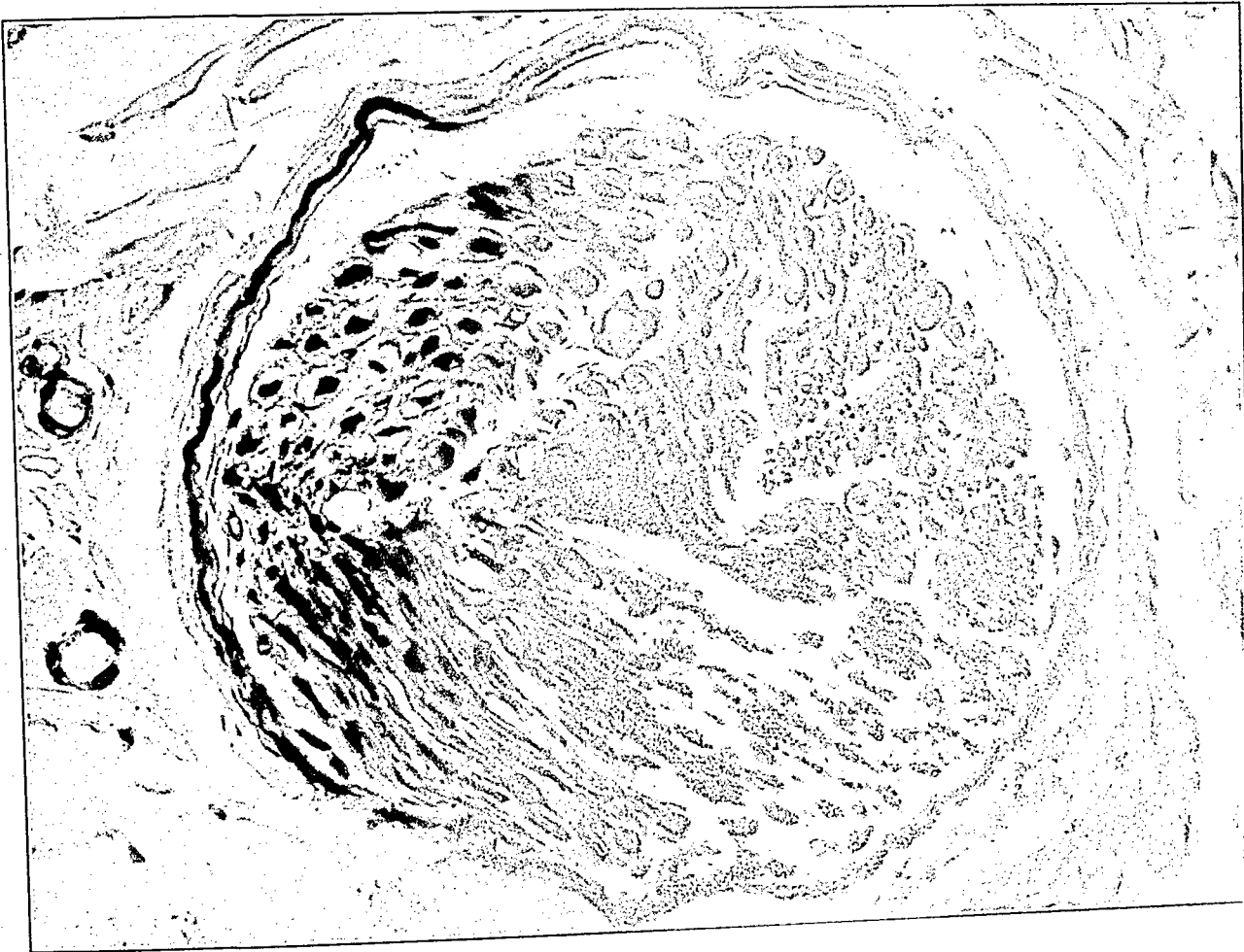
Lead Hematoxylin Stain for Axis Cylinders

PLATE 105

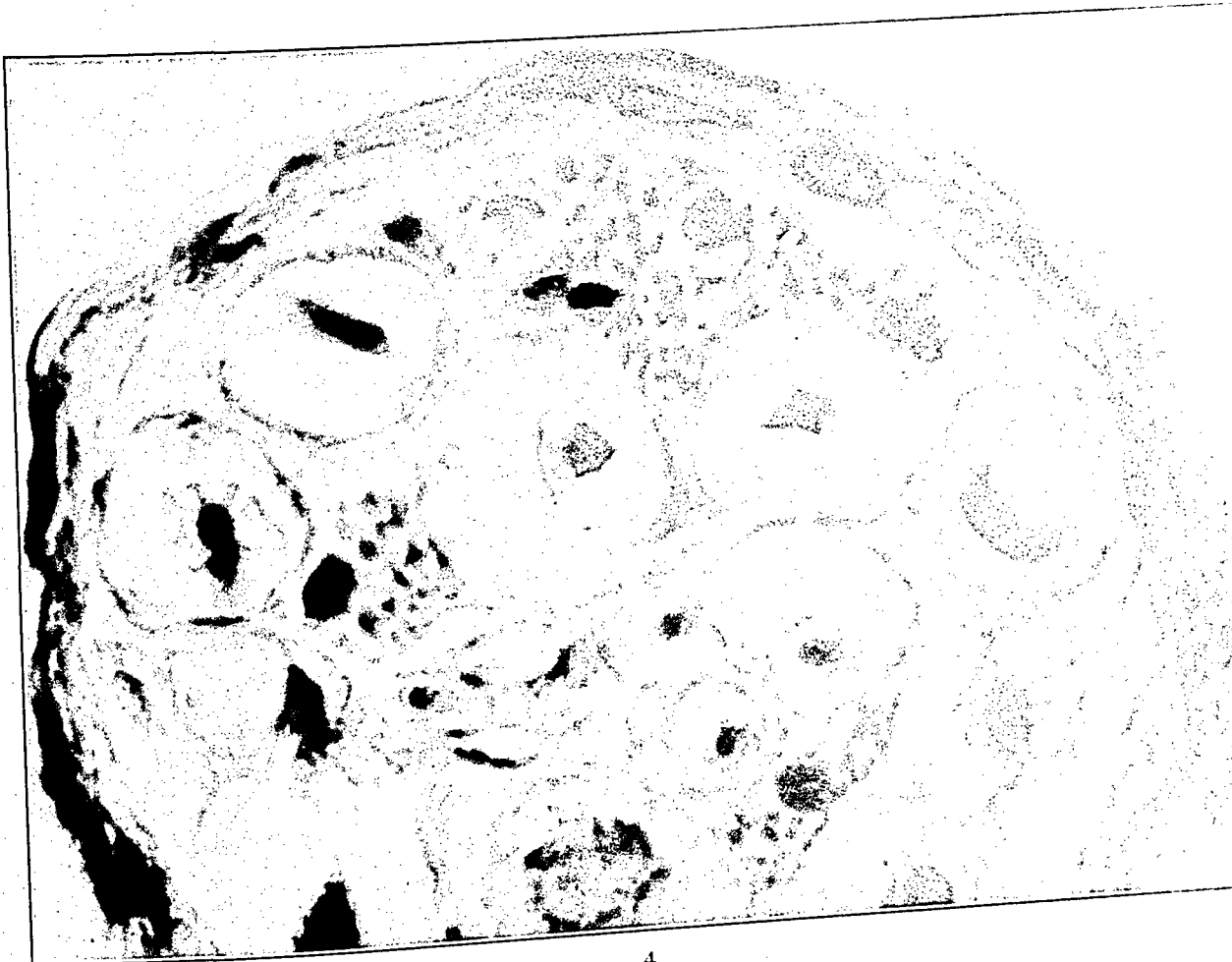
FIG. 3. Small branch of the splanchnic nerve showing myelinated and non-myelinated nerve fibers.  $\times 500$ .

FIG. 4. Still smaller branch of the splanchnic nerve showing myelinated and non-myelinated nerve fibers.  $\times 2250$ .





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Lead Hematoxylin Stain for Axis Cylinders

Mallory



# THE AMERICAN JOURNAL OF PATHOLOGY

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VOLUME XII

SEPTEMBER, 1936

NUMBER 5

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## THE NATURE AND SIGNIFICANCE OF THE STRUCTURAL CHANGES IN THE LUNGS IN MITRAL STENOSIS \*

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### INTRODUCTION

The lesions in cardiac disease are usually located in the left side of the heart, and it is the dysfunction of this side that is responsible for the majority of instances of circulatory failure. Ever since Corvisart's and Hope's contributions, morphologists have advocated a somewhat simplified mechanical "back pressure" concept of congestive failure. From time to time other explanations of circulatory failure have been offered, but the "back pressure" theory has received adequate support through the more recent physiological and chemical investigations. These studies have revealed that it is the disturbance of the pulmonary circulation that is the center of the problem of congestive failure.<sup>1, 2</sup> It is to the physiological and morphological changes within this circuit that the majority of the clinical manifestations are referable. While the study of the human pulmonary circulation during life has only recently become feasible, the effect of persistent passive congestion on the structure of the lungs has long been recognized and known under the term "brown induration." Furthermore, the pathological changes in the larger pulmonary arteries are familiar and are routinely looked for at autopsy, particularly in cases of mitral stenosis.

\* This investigation was aided in part by a grant from the Josiah Macy, Jr., Foundation.  
Received for publication February 17, 1936.

contained fibrous tags. The cut section presented a rather unusual picture. The lower halves of both lungs were gray and somewhat firm and dense, though crepitant; they contained little blood. The upper halves were red and intensely engorged (Fig. 2). Over the upper half and middle portion of the right lung there were several small infarcts, one of which was located at the tip of the apex. The trachea and bronchi were dull brownish red. The pulmonary artery was somewhat dilated and was of normal elasticity, but the intima revealed numerous, rather soft, yellow atheromatous patches.

The *liver* weighed 1460 gm. The surface was slightly granular. The vascular markings were intensified.

The rest of the postmortem examination, except for evidence of passive congestion, was irrelevant.

*Anatomical Diagnoses:* Rheumatic heart disease with an unusually high degree of stenosis and calcification of the mitral valve; pulmonary congestion and edema of the upper half, and induration of the lower half of the lungs; small pulmonary infarcts; right hydrothorax; obliterative healed left pleuritis; ascites; passive congestion of the liver; edema of the gall bladder; chronic cystitis and vaginitis.

### *Microscopic Description*

In describing the pathological changes in the *lungs*, their various component parts will be taken up separately:

1. *Alveolar Walls:* The severity of the lesions varied with the different lobes and portions of the lobes. In general, the lower lobes showed the most marked changes, particularly the lower parts of these lobes. Also, the lower portions of all the lobes were involved more markedly than the upper.

The least severe change noted was a marked dilatation of the capillaries, the diameters of their lumens being five or six times that of a red cell. In regions where the lesions were more advanced, not only were the capillaries dilated but they also appeared increased in number. This apparent increase may well have been due in part to increased length or to tortuosity. Often such capillaries presented an aneurysmal dilatation and bulged out into the alveolar space (Fig. 5). Accompanying this stage, the capillary basement membrane frequently showed slight thickening, but the alveolar basement membrane was normal.

A greater degree of change consisted in an increase in thickness of the capillary basement membrane and also of the interstitial collagen; the alveolar basement membrane even at this stage was normal. The capillaries here were dilated but showed less herniation into the alveolar spaces (Fig. 6).

Where the process had advanced further there was a definite increase in the interstitial connective tissue, and the capillary basement membrane was markedly thickened while the alveolar basement membrane remained normal. At this stage the capillaries were usually separated by the interstitial collagen from the alveolar space, which tended to be lined with cuboidal cells (Fig. 7). These cells often contained fat vacuoles. Thus the capillaries were separated from the alveolar space by structures of considerable thickness. Finally, the capillaries in such thickened walls became small, and in some none could be made out. Even in these advanced stages the alveolar basement membrane was never thickened, in contrast to the capillary basement membrane. These advanced structural changes were common findings in the lower third of the lungs.

Edema of the interstitial tissue of the wall was not uncommon. This was evidenced by separation of the component parts by fluid, with resulting thickening of the wall. The alveolar basement membrane was ballooned outward and it was in this condition that this membrane could best be recognized (Figs. 8 and 9). Such interstitial edema was by no means always accompanied by edema of the alveolar spaces.

The elastic tissue showed no definite changes.

There was often an infiltration of lymphocytes and macrophages in the thickened walls, and diapedesis of red blood cells into the walls was frequently seen. The alveolar spaces contained varying numbers of pigmented macrophages, and also edema fluid. In some areas emphysema was present. This led to narrowing of the alveolar wall with resultant diminution in the capillary lumens, often of such a high degree that a single red cell could barely pass through. In some such walls there was thickening of the collagen, indicating that congestion had existed here before the emphysema developed (Fig. 10).

A section through an infarct showed a picture, the significance of which will be discussed later. The parenchyma adjacent to the

infarct exhibited the most marked changes described above; *i.e.* great thickening of the alveolar walls with the air spaces lined by cuboidal epithelium. The alveolar walls in the infarcted area, on the other hand, showed slight if any increase in collagen and were

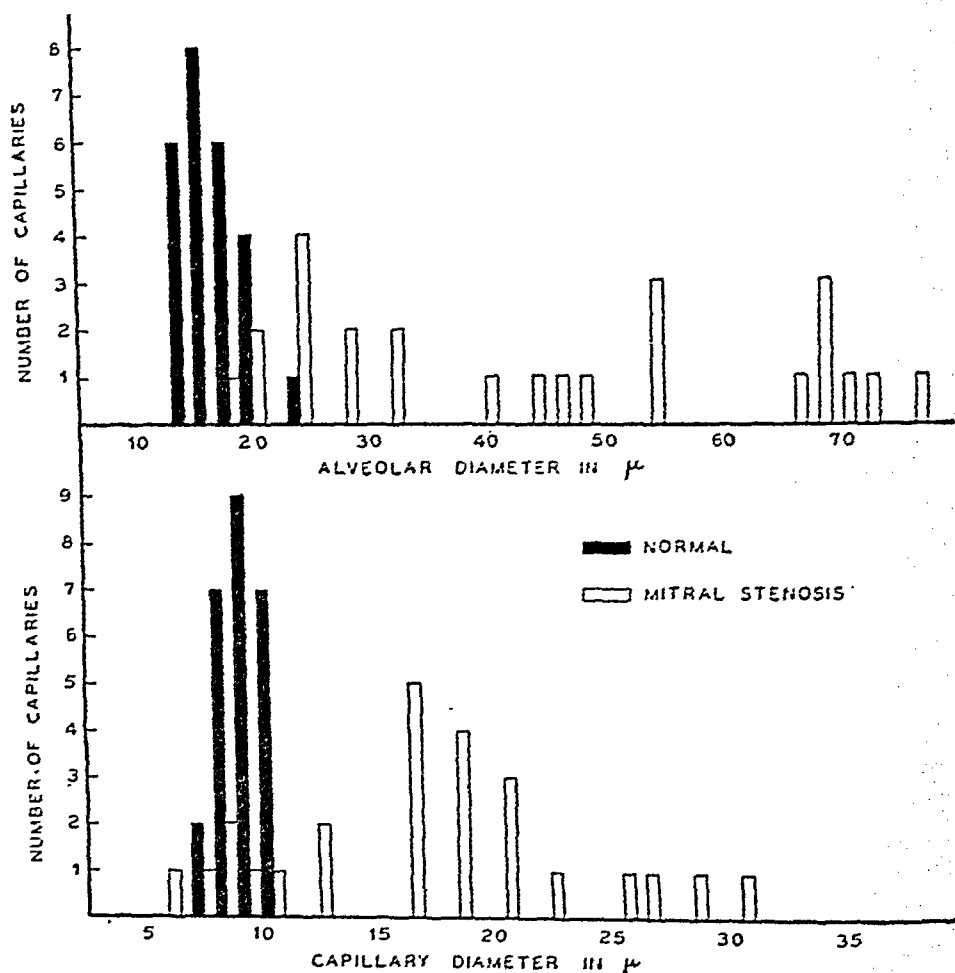


CHART 2

Sample of comparative measurements of the diameters of 25 alveolar walls and of corresponding capillary lumens of the normal lung and of the lung in advanced mitral stenosis

essentially normal as far as the connective tissue content of their walls was concerned. The condition of the smaller arteries and arterioles in the infarcted area could not be determined, since all cellular elements were, of course, completely necrotic.

The diameters of several hundreds of capillary lumens and of alveolar walls have been measured. Table I shows examples of such

measurements. There was usually a striking increase in both of these measurements, but the changes within the same alveolus did not parallel each other. Over the basal portion of the lungs, capillaries of normal diameter (7 to 9 microns) were often embedded centrally or eccentrically in alveolar walls of 40 to 60 microns in thickness, containing collagen staining intensely blue with the aniline blue stain. Chart 2 presents comparative measurements of the diameters of the alveolar walls and of the corresponding capillary lumens.

An attempt was made also to count the number of capillary lumens visible along the cross-section of an average sized alveolus. Chart 1 presents sample data of such counts in a normal lung, in a case of simple pulmonary basal congestion without heart disease, and in the case with mitral stenosis. As indicated, three to four times as many capillaries were visible in the presence of pulmonary congestion. It is of interest that the increase in the simple pulmonary congestion was of the same order of magnitude as in the case with mitral stenosis with intense active and passive congestion. Obviously, the number of capillary lumens visible along the cross-section of the alveolus does not correspond with the number of capillaries within the alveolus. If a capillary is tortuous the section may contain several cross-sections of the same capillary. Such counts as are presented in Chart 1 indicate, nevertheless, that in the normal lung (postmortem) the majority of the capillaries are not filled with blood unless there is active or passive congestion. Judging from the histological appearance of the capillaries and from the capillary counts, in the case of mitral stenosis not only were all the available pulmonary capillaries filled with blood, but they were stretched to their maximal capacity.

2. *Arteries:* (A) *Large Arteries:* These vessels showed marked thickening of the intima as a result of proliferation of connective tissue, which in its deeper portions often contained large, lipoid-filled macrophages. Reduplication and splitting of the internal elastic laminae were present. Thrombi undergoing various stages of organization were common.

(B) *Medium Sized Arteries:* These presented an unusual picture. The early lesion apparently consisted of a subendothelial deposition of fibrin with an associated proliferation of the lining endothelial cells (Fig. 11). Accompanying this stage was an increase in the

intimal connective tissue, in which appeared a number of capillaries. In the late stage the lumen was represented by narrow endothelial-lined spaces and the intima by vascular connective tissue (Fig. 12).

(C) *Small Arteries*: Marked intimal thickening due to increase in connective tissue and also some medial hypertrophy characterized the changes in this group. The alterations of the intima were the more striking, and resulted in a disproportion between the thickness of this layer as compared with that of the media.

(D) *Arterioles*: The walls of these vessels showed a high degree of thickening with apparent diminution in the diameters of their lumens. This thickening was due to a concentric proliferation of the cells making up their walls (Figs. 13 and 14). Between these cells lay delicate bands of collagen. In addition, a rare vessel undergoing necrotizing arteriolitis could be found (Fig. 15). Such arterioles showed blurring of the outlines of their walls with karyorrhexis of the nuclei and diapedesis of red cells. This type of lesion was also noted at the periphery of an infarct of some age. Hyalinization of the arterioles was not seen.

3. *Veins*: The only change of note was a thickening and hyalinization of the surrounding collagen.

4. *Interstitial Connective Tissue*: Edema of the septa was present in a marked degree without an associated edema of the alveolar spaces (Fig. 16). There were several foci of myelocytes and nucleated red cells in the perivascular and pleural connective tissue. The pleura showed marked increase in connective tissue.

*Heart*: There was some fibrous thickening of the epicardium with an infiltration of lymphocytes. In the myocardial connective tissue a rare Aschoff body and a few lymphocytes were noted. There was a minimal amount of scarring. Branches of the coronary arteries were thickened.

*Liver*: Hemorrhagic central necrosis, both old and recent, was present.

The other organs revealed nothing remarkable on postmortem examination.

### Summary

The main features of this case may be summarized as follows:

1. An unusually advanced degree of mitral stenosis was associated with attacks of dyspnea, orthopnea and pulmonary edema.



During the last 4 weeks of life the patient exhibited a constant state of pulmonary edema and congestion. Clinical evidence of failure of the peripheral circulation appeared only as a terminal manifestation.

2. Because of the intense basal pulmonary congestion and edema and probable secondary infection, a severe degree of induration of the lower half of the lungs developed. This induration consisted mainly in a marked degree of thickening of the alveolar walls due to increased connective tissue and to a change of the flat epithelial layer into a cuboidal one. As a result of these changes the pulmonary capillaries became compressed; many of them were displaced into the middle portion of the alveolar wall and became surrounded by thick layers of collagen. Several of the alveoli contained no capillaries.

3. As a result of the above changes over the lower half of the lungs the congestion over the upper part of the lungs became intensified. Here, including even the apical area, the capillaries became widely dilated, many of them showing aneurysmal dilatation and bulging into the alveolar spaces. The capillary basement membrane showed various degrees of thickening, in contrast to the unchanged alveolar basement membrane. Separation of the two basement membranes by pericapillary edema was frequently observed.

4. There was atherosclerosis of the larger pulmonary arteries, healing and healed rheumatic arteritis of the medium sized arteries, and hyperplastic arteriolosclerosis and rarely necrotizing arteriolitis of the arterioles. It was particularly significant that both the frequency and the degree of the arteriolar changes increased from the apex towards the base.

5. The main changes in the veins consisted in increased connective tissue surrounding them.

6. Edema of the interlobular septa occurred independently of alveolar transudation.

### *Findings in Other Cases*

In order to ascertain the significance of the pulmonary and vascular lesions observed in the case described, we have studied in a similar detailed manner 9 additional cases with mitral stenosis of rheumatic origin. Four cases in this group showed alveolar and vascular changes similar to those found in the first case but without rheu-

matic arteritis. In the remaining 5 cases the lesions were much slighter, consisting mainly of some thickening of the alveolar wall with a moderate degree of increase in the collagen and with distended capillaries over the lower portion of the lungs; there were no changes in the small arteries and the arterioles.

A comparison of the clinical and gross morphological characteristics of the 5 cases with advanced pulmonary vascular and parenchymatous lesions with those of the 5 cases with mainly intense pulmonary passive congestion revealed the following features: Four of the 5 subjects with marked pulmonary and vascular changes were young, the ages varying between 26 and 36 years. The age of the 5th subject was 58 years. The first rheumatic infection and the discovery of heart disease in each case dated back to childhood but in all cases manifestations of a pronounced degree of cardiac failure appeared within 1 to 3 years of death. The circulatory failure was characterized mainly by intense cyanosis, complete physical disability associated with progressive and continuous severe dyspnea and orthopnea, precordial oppression and pain, with or without radiation, and later superimposed attacks of paroxysmal dyspnea and hydrothorax. The patients complained of periodic attacks of severe cough, "bronchitis" and hemoptysis. All these symptoms were present at first without evidence of peripheral congestive failure, such as venous engorgement, ascites, enlargement of the liver, jaundice, and edema of the lower extremities. Such manifestations developed relatively late. Once advancing cardiac failure appeared the patients responded poorly to treatment. The cardiac rhythm was regular or auricular fibrillation. The first cardiac sound was loud and the pulmonary second sound accentuated and reduplicated. Other cardiac manifestations of mitral stenosis were also present. The electrocardiograms in three instances revealed high P waves. The contour of the X-ray picture revealed mitral bulging and varying degrees of increased pulmonary conus. The clinical features of these patients corresponded in several aspects to those described by Held, Goldbloom and Lieberman,<sup>5</sup> but the degree of cyanosis and respiratory difficulties were more intense.

Postmortem examination revealed a slit shaped, narrow mitral orifice in 4 cases. In the 5th case there was stenosis admitting the tip of the little finger. In all 5 cases the narrowed and distorted mitral leaflets were sclerosed and calcified and the chordae tendineae

were shortened and thickened. Acute rheumatic verrucous endocarditis was not present. The other valves were normal except in 2 cases in which the edges of the aortic cusps were "rolled" and slightly thickened. The weight of the heart was slightly or moderately increased. The left ventricle was of normal size and thickness, but the other chambers of the heart showed pronounced dilatation as well as thickening. In each of the 5 cases mural auricular thrombi were present. One or both of the pleural cavities contained from 1 to 2 liters of fluid. The peritoneal cavity was either filled with fluid or normal. The large pulmonary arteries showed a slight degree of sclerosis. The bases of both lungs in each of the 5 cases were gray, the consistence was increased and firm, and they contained a decreased amount of air. The upper portions of the lungs were red and congested and contained one or more small red infarcts. The severity and extensiveness of the basal induration and congestion of the upper portions varied, but in none of the 4 cases was the degree as severe as in Case 1.

The ages in the other group of 5 cases were more advanced. The onset of the rheumatic infection dated back to youth, and the duration of heart disease was longer. The onset and the progress of the symptoms of advancing cardiac failure were gradual, and there was a lesser degree of dyspnea, orthopnea and suffering in general than in the group previously described. These patients did not suffer from paroxysmal dyspnea. Cyanosis was either slight or absent. The clinical manifestations of pulmonary and peripheral congestion appeared simultaneously.

Postmortem examination showed a healed, scarred and calcified mitral valve admitting the tip of one finger. The chordae tendineae were shortened and thickened. In addition to the mitral stenosis, 1 case showed slight aortic stenosis, 1 a moderate degree of tricuspid stenosis and 1 aortic insufficiency. The degree of cardiac hypertrophy was greater and the auricular dilatation less than in the previous group.

The pleural cavities contained no fluid or only moderate amounts; the peritoneal cavity was either partially or completely filled with fluid. The large pulmonary arteries showed a moderate degree of sclerosis. The lungs exhibited basal congestion.

In addition to the detailed study of these 10 cases, the routine histological sections of 13 cases with mitral stenosis of varying de-

gree associated with other types of rheumatic cardiac involvement were examined, but sections of only 2 of these cases exhibited diffuse sclerosis of the small arteries and of the arterioles. A certain degree of thickening of the alveolar wall was frequently observed.

### *Control Cases*

It is of interest that sections of the lungs from 5 cases of congenital cardiac septal defect, 12 cases of congestive circulatory failure of luetic and hypertensive origin, 15 cases showing pulmonary emphysema, 1 case of marked kyphosis and sclerosis of the larger pulmonary arteries, 20 cases of thrombosis or embolism of the pulmonary arteries, 19 cases showing chronic interstitial pneumonitis, and 3 cases of pulmonary fibrosis of non-cardiac origin failed to reveal pulmonary vascular and parenchymatous changes similar to those in the 5 cases that showed advanced mitral stenosis.

Through the courtesy of Dr. Tracy B. Mallory we have also examined the slides from a case diagnosed as Ayerza's disease (No. 6248). In this case, however, the small vessels were not involved and the alveolar structure was normal. Intimal proliferation and occlusion of several of the larger vessels were the main findings.

### DISCUSSION OF PATHOLOGICAL FINDINGS

The changes in the alveolar walls described above are in essential agreement with those reported by zu Jeddelloh.<sup>3</sup> Such changes are presumably the result of long continued hypertension, stagnation and edema. As a result of the increased intravascular pressure there occurs first a dilatation and apparent elongation of the capillaries. Following this there is a thickening of the capillary basement membrane, and this finally is followed by an increase in the interstitial collagen of the alveolar wall. This latter change we consider the result of interstitial edema.

As a result of the increased connective tissue and the consequent separation of the capillaries from the alveolar spaces, the involved alveoli are no longer capable of functioning. This is indicated by the cuboidal type of cells lining the air sacs. Function is also interfered with by the interstitial edema which fills and increases the space between the capillaries and air sacs. Furthermore, at various areas emphysema results in a marked diminution in the caliber of the cap-

illary bed, due to stretching with resulting narrowing of the alveolar walls. The diameter of the capillaries in such thin alveolar walls is frequently less than normal, and this narrowed capillary bed provides an increased resistance to the blood flow. It is of interest to note that emphysema occurred in some regions where apparently chronic congestion had existed previously, as indicated by the increased amount of collagen in the alveolar walls.

The vascular changes varied with the size of the blood vessels in question. The larger arteries showed proliferative changes in the intima with, in addition, the occurrence of lipoid-filled macrophages. The intimal proliferative changes consisted of increased connective tissue with splitting and reduplication of the internal elastic laminae. Such changes have for many years been ascribed to the effect of hypertension, and in our opinion are rightly so interpreted, since analogous lesions are seen in the kidney in cases of hypertension.

The changes in the medium sized arteries described in our first case are to be regarded as probably of rheumatic origin, in view of the studies of VonGlahn and Pappenheimer.<sup>6</sup> We observed such lesions in none of our other cases. In them, the medium sized and smaller arteries showed marked fibrous thickening of the intima of such a degree that often the intima was thicker than the media. There was also some hypertrophy of the media.

The arterioles presented the picture of hyperplastic arteriosclerosis (productive endarteritis), *i.e.* thickening of the walls due to concentric cellular proliferation, giving an onion-like layer of cells in the walls. This vascular change especially aroused our interest because of its similarity to the arteriolar changes in the kidney in malignant hypertension. This similarity was further emphasized by the occurrence — rare, it is true — of necrotizing arteriolitis. This lesion, in addition to being present in scattered areas in the lung, was found also in the vicinity of an infarct, a fact the significance of which for renal infarcts has been discussed by Klemperer and Otani<sup>7</sup> and more recently by Kimmelstiel and Wilson.<sup>8</sup> No hyaline degeneration of the arterioles such as is seen in the kidney, pancreas and adrenals in benign hypertension was noted in the lungs in our series.

In reviewing the vascular changes in the lung described above, one is struck by their similarity to the changes observed in the kidney in malignant hypertension. In both organs the larger arteries

show proliferative changes in the intima. The arterioles show hyperplastic arteriosclerosis and in addition necrotizing arteriolitis. In view of this, it seemed of interest to determine approximately the dimensions of the vessels showing these various changes in the two organs under discussion. For this purpose, the external diameters of the vessels were measured. In both organs it was found that the great majority of vessels showing hyperplastic arteriosclerosis and arterionecrosis measured less than 100 microns, while those showing intimal proliferative changes measured more than 150 microns.

The vascular changes in both organs lead to serious interference with their functional units, *i.e.* the glomerulus and the alveolar wall. These two structures have in common certain anatomical and physiological functions. Both are made up of capillaries, surrounded by a basement membrane which is covered by epithelium. As a result of vascular alterations, both units undergo changes which eventually result in the complete loss of function. In the kidney the glomerulus becomes a hyalined scar; in the lung the alveolar wall a broad band of rather avascular connective tissue.

Finally, we feel that the histological picture found in the infarct in Case 1 is important from the point of view of the length of time necessary to produce the pulmonary changes we have described. As pointed out in the microscopic description, the alveolar walls in the infarct showed no increase in connective tissue, while the adjacent walls exhibited a marked degree of thickening and the alveolar epithelium was cuboidal in type. There are apparently two explanations of the above picture: (1) the infarct involved normal tissue, and (2) the infarct occurred before the parenchymatous changes had taken place. Against the first suggestion is the fact that all the surrounding parenchyma is pathologically altered and it seems improbable that there should be a directly contiguous area of normal tissue, since the process in any one given area tends to be uniform and diffuse. If the second explanation, which seems the more probable, is correct, then we have definite evidence that the changes in the alveolar walls can occur in a comparatively short period of time, for the infarct shows but little signs of healing. In our estimation the duration was weeks rather than months.

## CLINICAL AND PHYSIOLOGICAL SIGNIFICANCE OF STRUCTURAL ALTERATIONS IN THE LUNGS

The clinical manifestations of failure of the pulmonary circulation are believed to depend on a dynamic alteration of the blood flow secondary to the back pressure effect of cardiac lesions or dysfunction in the left side of the heart. The physiological changes manifest themselves primarily in retardation and stagnation of the flow, which is more marked over the lower than over the upper portions of the lungs.<sup>2, 9, 10, 11</sup> The observations here presented, however, demonstrate that the circulatory engorgement can induce permanent structural alteration of various tissue components of the lung, which, in turn, can then be the source of disturbed functions, as well as of clinical symptoms and signs. *The nature of these structural changes is such that they interfere with the vital pulmonary function of gaseous exchange.* Over the lower half of the lungs the capillary basement membrane becomes thickened and deposition of a considerable amount of collagen separates the capillary lumen from the alveolar space. Not infrequently the flat, thin epithelial cells become thickened and cuboidal. The normal thickness of 1 to 3  $\mu$  of the alveolar tissue, through which oxygen and carbon dioxide have to diffuse, can change to a thickness of 30–50  $\mu$ . Over such pulmonary areas the blood flow is shunted without any appreciable degree of alteration of its gaseous contents. In advanced cases of "tight mitral stenosis," thickening of the alveolar wall with markedly dilated capillaries may be present not only in the lower but also, as has been shown, in the upper portion up to the apex. In spite of the fact that even in these high non-dependent areas the pericapillary collagen can be somewhat increased, both oxygen and carbon dioxide obviously must diffuse freely through these alveolar structures. Nevertheless, the fact that the diameters of the *distended capillaries* permit the simultaneous passage of from five to twenty or more red cells, instead of one or two, must be a significant contributory factor to the maintenance of arterial anoxemia. It is, however, of interest that study of the arterial blood in patients with circulatory failure often indicates no increase in the carbon dioxide content, even in the presence of a severe degree of anoxemia.<sup>12</sup> This difference in oxygen and carbon dioxide content must be explained by the greater diffusion coefficient of carbon dioxide. Liljestrand and

Sahlstedt<sup>13</sup> have found that carbon dioxide diffuses about forty times more rapidly than oxygen through the alveolar wall of the lung of the frog.

Patients with chronic heart disease, particularly with mitral stenosis, at times exhibit intense cyanosis regardless of subsequent myocardial improvement, as indicated by studies of hemodynamics. The structural alterations in the pulmonary architecture observed in this study suggest that if, as a result of long existing pulmonary engorgement, the structural alterations are permitted to develop, certain symptoms such as cyanosis and even dyspnea may persist regardless of the myocardial improvement established thereafter. The change in size and in the elastic properties of the lung tissue in these cases must represent, for the development of dyspnea and tachypnea, a stimulus to the nerve endings located in the lungs and in the pleura similar to that present in chronic emphysema or interstitial pneumonitis. The finding of thickened alveoli exhibiting increased amounts of collagen and other changes demonstrates that in the presence of chronic failure of the circulation not only physiological but also structural alterations contribute to the *stiffening of the lungs* ("Lungenstarre" of von Basch<sup>14</sup>).

Some of the patients whose lungs were studied exhibited rather profuse transient pulmonary hemorrhages. The rupture of the large bulging capillaries observed is an obvious source of such hemorrhages. Whether diseased arterioles rupture at times, we do not know.

We have been impressed in the past by the frequent lack of correlation between acute or chronic dyspnea and orthopnea, on the one hand, and such clinical signs as pulmonary râles, on the other hand. On postmortem examination one observes heavy lungs with only a moderate degree of hyperemia and no appreciable amount of edema within the air passages. The demonstration of the existence of *pericapillary edema* without intra-alveolar edema offers adequate explanation for such a situation, and the findings presented demonstrate also that a considerable amount of tissue fluid may accumulate around the capillary bed. Such a state of affairs must contribute to the patient's distress, and this type of edema, in contrast to other structural changes, should be amenable to therapeutic procedures. We have also observed not uncommonly the opposite situation, namely, the existence of *intra-alveolar edema* without capillary en-



gorgement and without alteration in the structure of the alveoli. In this type of pulmonary edema a primary alteration of the permeability must be the determining factor. Finally, pericapillary and intra-alveolar edema often coexist.

When both primary pulmonary *emphysema* and *heart disease* were present, the capillary bed usually was not dilated. In these cases, although the alveolar wall shows no thickening or only a slight degree of thickening, an increase in the collagen content of the alveolar wall has been demonstrated. Hence, here, too, the circulatory engorgement contributes through structural alterations to the further rigidity of the alveoli. The fact that in an emphysematous lung the already diminished capillary bed cannot dilate, represents loss of an important vascular reserve function of the lung, which is essential under certain types of stress. Loss of such reserve function must facilitate the development of pulmonary hypertension.

In attempting to throw light on the etiology of pulmonary arteriosclerosis, it seemed significant that the advanced vascular lesions were situated in the lower portions of the lungs, while in the upper portions sclerosis was not present or was but slight. This striking difference in the distribution of the vascular lesions must bear pertinently on the origin of the type of sclerosis described. The main differential characteristics of the functional state of the lower portions of the lungs as contrasted with those of the upper portions in the cases studied were: (1) higher capillary and arteriolar pressure; (2) slower blood flow and stagnation; and (3) pericapillary and intra-alveolar edema. It is therefore rational to conclude that the vascular changes observed are dependent on these three factors, or on chemical or morphological alterations which are secondary to these factors. It is of significance, also, that in malignant nephrosclerosis, high pressure, as well as stagnation, is present within the arterioles exhibiting sclerosis, as is indicated by injection methods.<sup>15</sup>

That combination of these three factors seems to be essential is shown by the fact that in pathological conditions where only one of the factors is present, arteriolar sclerosis, and particularly necrotizing arteriolitis are not observed. Thus the clinical and histological examinations of the upper portion of the lung in Case 1 have indicated markedly increased vascular pressure, but vascular sclerosis was not present. In the control group of pulmonary emphysema and congenital heart disease with hypertrophied right ventricle, sclerosis

was also absent, in spite of the fact that the pulmonary blood pressure was presumably high. Similarly, in a group of cases with long persisting chronic passive congestion and edema of cardiac origin, or with chronic pneumonitis and fibrosis, the vascular lesions were not present. On the other hand, it has been shown recently that long persisting pulmonary edema induced in the rat by the prolonged administration of oxygen under high barometric pressure can induce a type of pulmonary arteriolosclerosis consisting of a thickening and hyalinization of the walls with ultimate thrombosis of many.<sup>16</sup> It is of interest that in addition to engorgement and edema, the lungs of these animals also exhibited increased arterial pressure.<sup>17</sup> These studies are of particular interest because they rule out anoxemia as an etiological factor.

It would be of significance to be able to estimate the time element essential for the development of the arteriolar lesions described. It is therefore pertinent that when the patient in Case 1 entered the hospital the lung fields were essentially of normal density, as indicated by X-ray examinations, and the marked degree of density of the lower half of the lungs developed during a period of  $2\frac{1}{2}$  months. It was also of significance that the red infarcted areas contained no thickened alveoli, while the non-infarcted areas at a corresponding level exhibited all grades of parenchymatous changes. As these infarctions were not older than 1 or 2 months, the advanced pulmonary lesions must have developed within the same period of time. That in malignant nephrosclerosis similar types of lesions can develop in the kidney within a period of the same order has been actually demonstrated by us in a comparative study of the two kidneys of the same case obtained at an interval of 67 days.<sup>18</sup> Finally, it has been shown that pulmonary arteriolosclerosis and an increase in the interstitial collagen can be induced experimentally within 30 to 40 days.<sup>16</sup>

Advanced ("tight," "non-regurgitant," "buttonhole") mitral stenosis is but one type of lesion that can lead at times to the combined presence of the three pulmonary factors mentioned. In cases with chronic pulmonary emphysema or congenitally increased pulmonary vascular resistance in which repeated attacks of pneumonitis or other types of change associated with edema develop, the three responsible factors are particularly apt to coexist and hence pulmonary arterial and arteriolar sclerosis are expected to occur.

Such may indeed be the etiological mechanism in instances described as primary pulmonary sclerosis of "Ayerza syndrome."

The 5 cases of hyperplastic and necrotizing arteriolitis exhibited intense dyspnea, orthopnea, tachypnea, cyanosis and precordial oppression and pain. Superimposed on these difficulties were attacks of severe cough, hemoptysis and cardiac asthma with transient intra-alveolar edema. The pulmonary second sound was loud and reduplicated. These manifestations are indicative of unusually high pulmonary pressure. The postmortem examination likewise suggested high pressure throughout the pulmonary circuit.

It has also been shown in this study that these cases of advanced mitral stenosis exhibited vascular changes quite similar to those found in the arterioles of the larger circuit, particularly in the kidneys of patients with malignant arterial hypertension. It has also been stressed earlier that a number of similarities exist between the function and structure of the glomerulus and alveolus. While in the presence of advanced mitral stenosis the pulmonary vascular system is involved and the arteriolar system of the larger circuit is normal, in cases of uncomplicated malignant hypertension of the larger circuit the situation is reversed, and the vascular system of the pulmonary circuit is normal. These clinical and physiological considerations, together with the morphological findings here presented, indicate a close similarity existing in the pulmonary circuit in the group of cases of mitral stenosis, and in the larger circuit in the group designated as malignant hypertension with malignant nephrosclerosis.<sup>18</sup> Hence these advanced cases of mitral stenosis can be considered as exhibiting the clinicopathological syndrome of *pulmonary hypertension with malignant sclerosis*. This designation does not imply that the syndrome is always clear-cut, or that clinically it can always be sharply differentiated from the syndrome of pulmonary hypertension without advanced or malignant sclerosis. In this respect, too, the problem of the malignant sclerosis of the two vascular circuits is identical.

Because the mechanical and circulatory factors active in this type of mitral stenosis leading to malignant pulmonary sclerosis are known to a large extent, further studies on the origin of these pulmonary vascular lesions may well throw light on the etiology of vascular changes in malignant hypertension and malignant nephrosclerosis.

## SUMMARY AND CONCLUSIONS

1. The changes in the blood vessels and alveolar walls in the lungs in cases showing an advanced degree of rigid mitral stenosis associated with intense failure of the pulmonary circulation are presented, and the structural alterations found are compared with those observed in other types of mitral stenosis and in a control group with varied types of cardiac and pulmonary disease.

2. The lesions in the pulmonary vessels consisted of (a) intimal thickening of the arteries, and (b) hyperplastic arteriolar sclerosis and arteriolar necrosis.

3. The changes in the alveolar walls consisted of (a) marked dilatation of the capillaries, (b) increase in the thickness of the capillary basement membrane, (c) increase in the interstitial tissue (collagen), (d) interstitial pericapillary edema, and (e) a tendency of the flat epithelial cells to become cuboidal in shape.

4. The normal thickness of 1 to 3  $\mu$  of alveolar tissue through which oxygen and carbon dioxide have to diffuse can increase up to a thickness of 30-50  $\mu$ .

5. Even in the presence of an advanced degree of thickening of the alveolar wall and of the capillary basement membrane, the alveolar basement membrane remains normal.

6. With progressive pulmonary engorgement, first the visible capillaries increase in number, and only later do they dilate. Often the capillaries become displaced and are separated from the alveolar surface by a considerable degree of edema or by thick layers of collagen.

7. Pericapillary and intra-alveolar edema frequently develop independently.

8. Permanent structural alterations in the lungs caused by circulatory failure interfere with the gaseous exchange, partly through altered permeability of the alveolar wall, and partly as a result of the simultaneous passage through the individual capillaries of numerous columns of red cells, instead of a single red cell.

9. In the causation of the pulmonary arterial and arteriolar lesions, an important rôle is played by the prolonged combined presence of (a) high intravascular pressure, (b) stagnation of blood flow, and (c) edema.

10. Evidence is presented that advanced vascular lesions in the

pulmonary, as well as in the larger circulation, can develop within about 2 months.

11. The clinicopathological syndrome of pulmonary hypertension with "malignant" sclerosis is described and the similarity between this condition and arterial hypertension with malignant nephrosclerosis is discussed.

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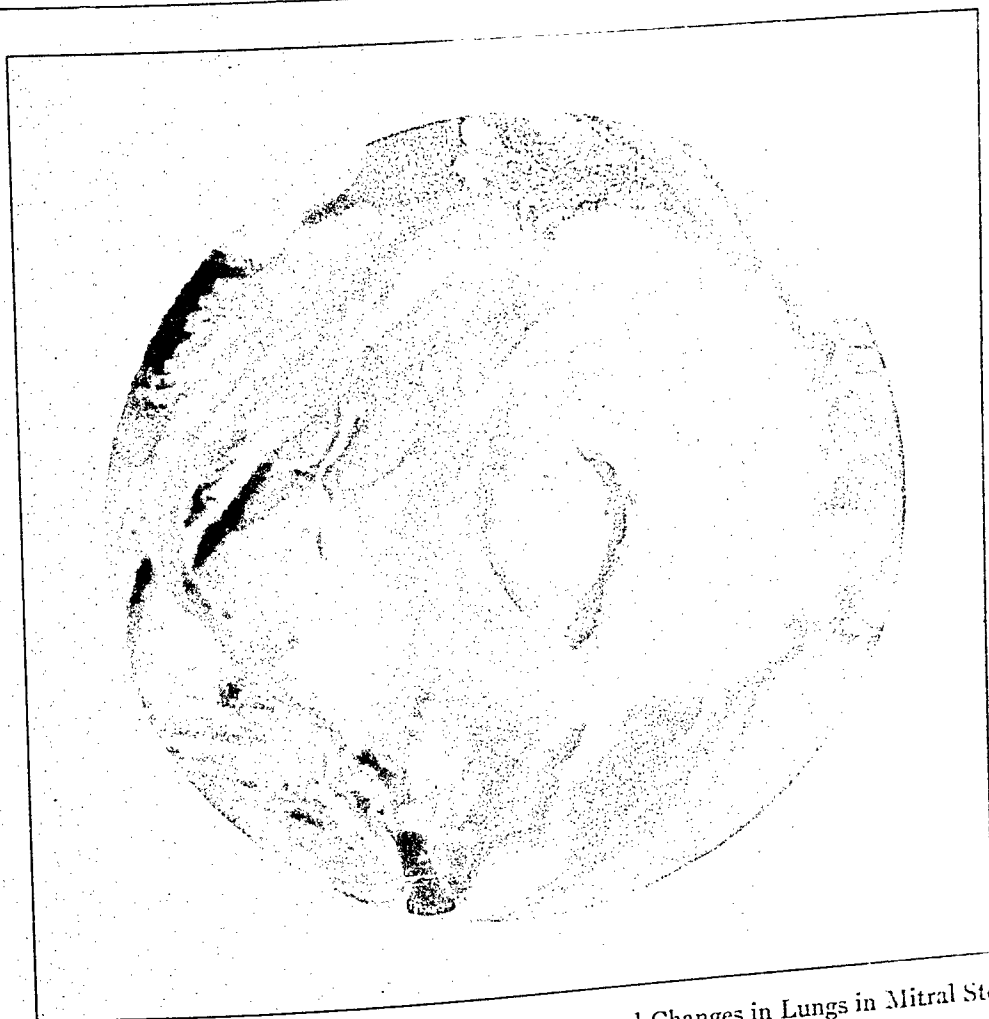
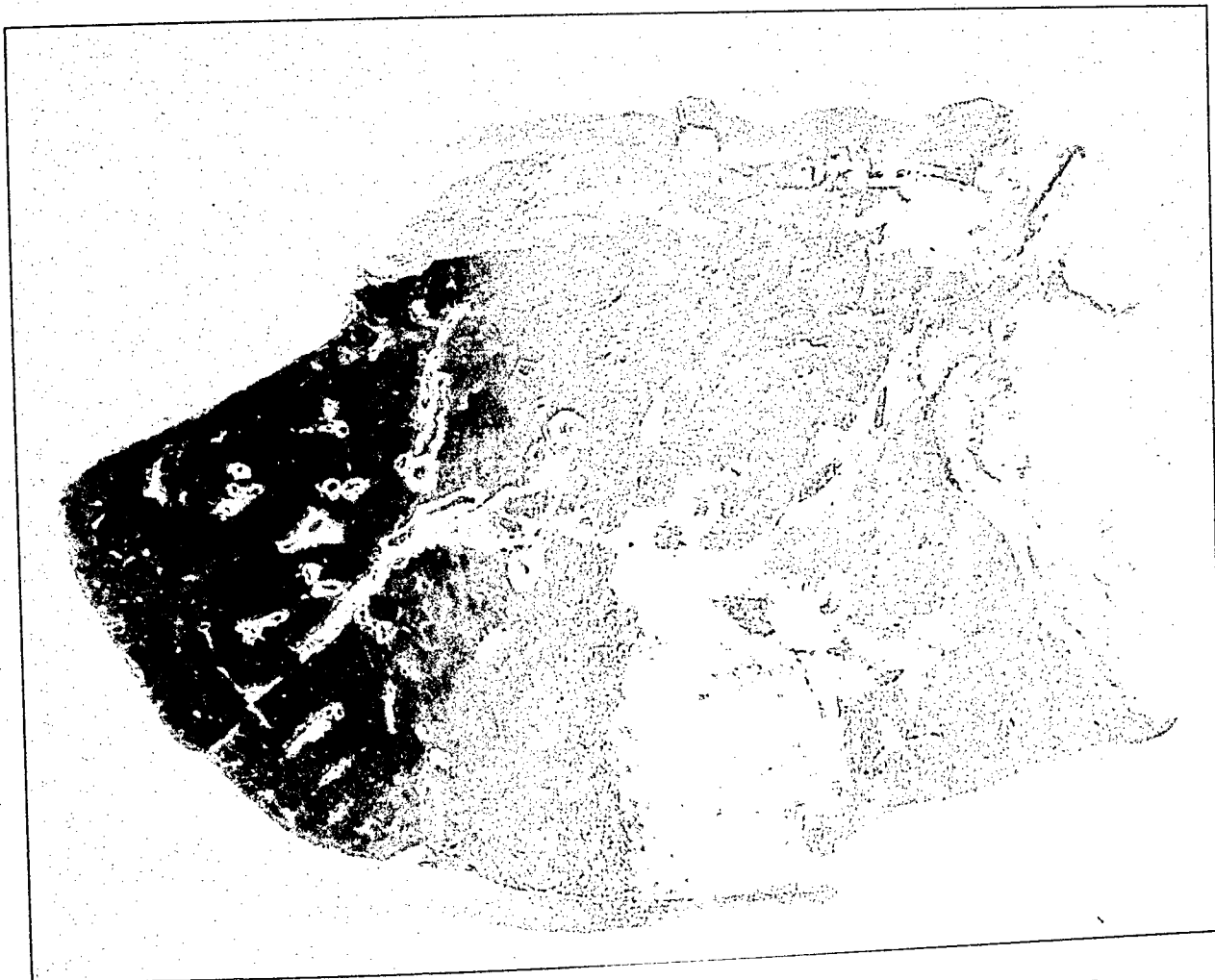
## DESCRIPTION OF PLATES

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### PLATE 106

FIG. 1. Mitral valve from Case 1, viewed from above. Note narrow, slit-like, rigid orifice.

FIG. 2. Lung from Case 1. Upper half red and congested, lower half gray and indurated.



Parker and Weiss

Structural Changes in Lungs in Mitral Stenosis

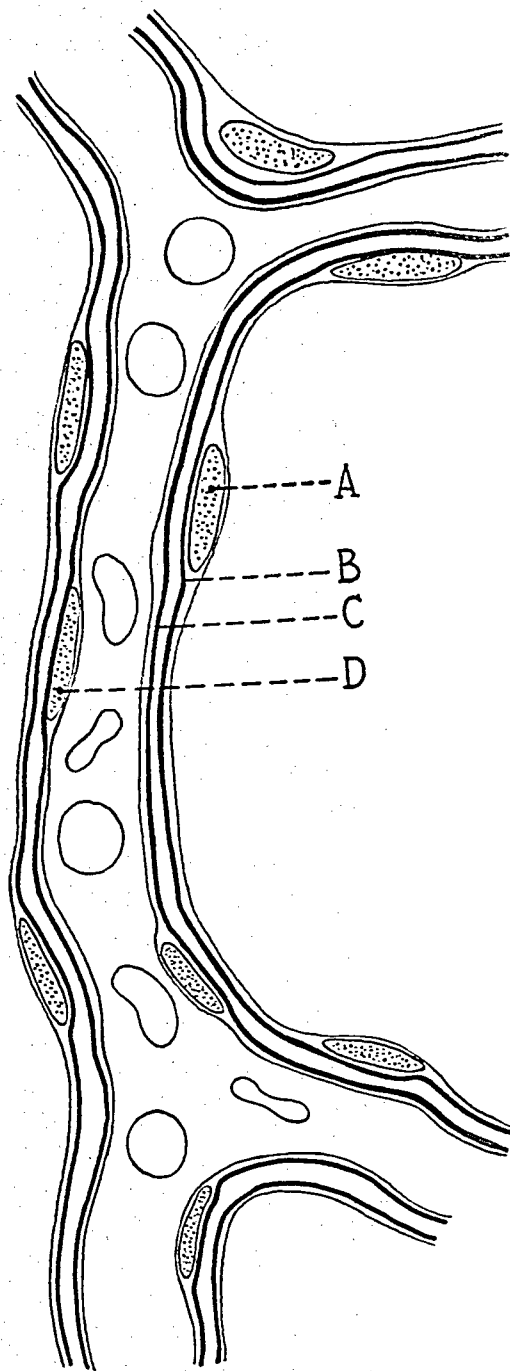
PLATE 107

FIG. 3. Diagrammatic drawing of structure of alveolar wall, emphasizing the location and relations of the basement membranes.

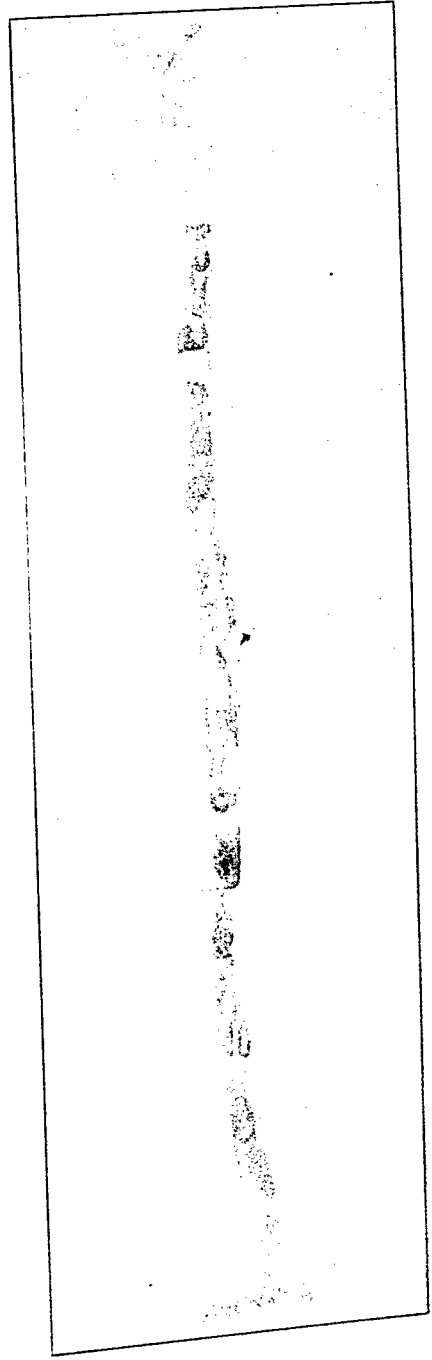
- A = epithelial cell lining alveolus
- B = alveolar basement membrane
- C = capillary basement membrane
- D = endothelial cell lining capillary

FIG. 4. Normal alveolar wall. Capillary sufficiently wide to admit passage of a single red cell. Alveolar and capillary basement membranes appear as a single thin line. Aniline blue stain.  $\times 670$ .





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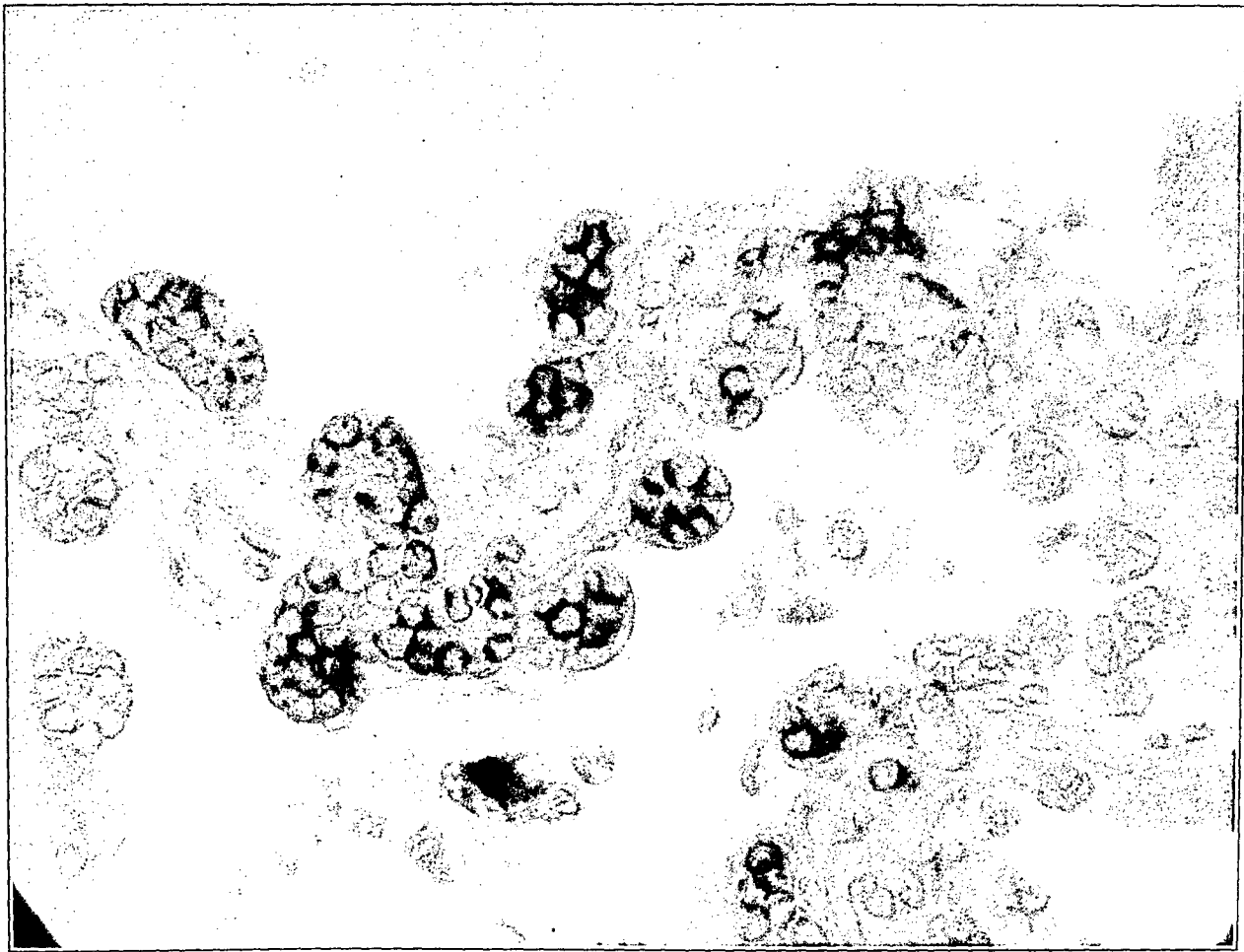


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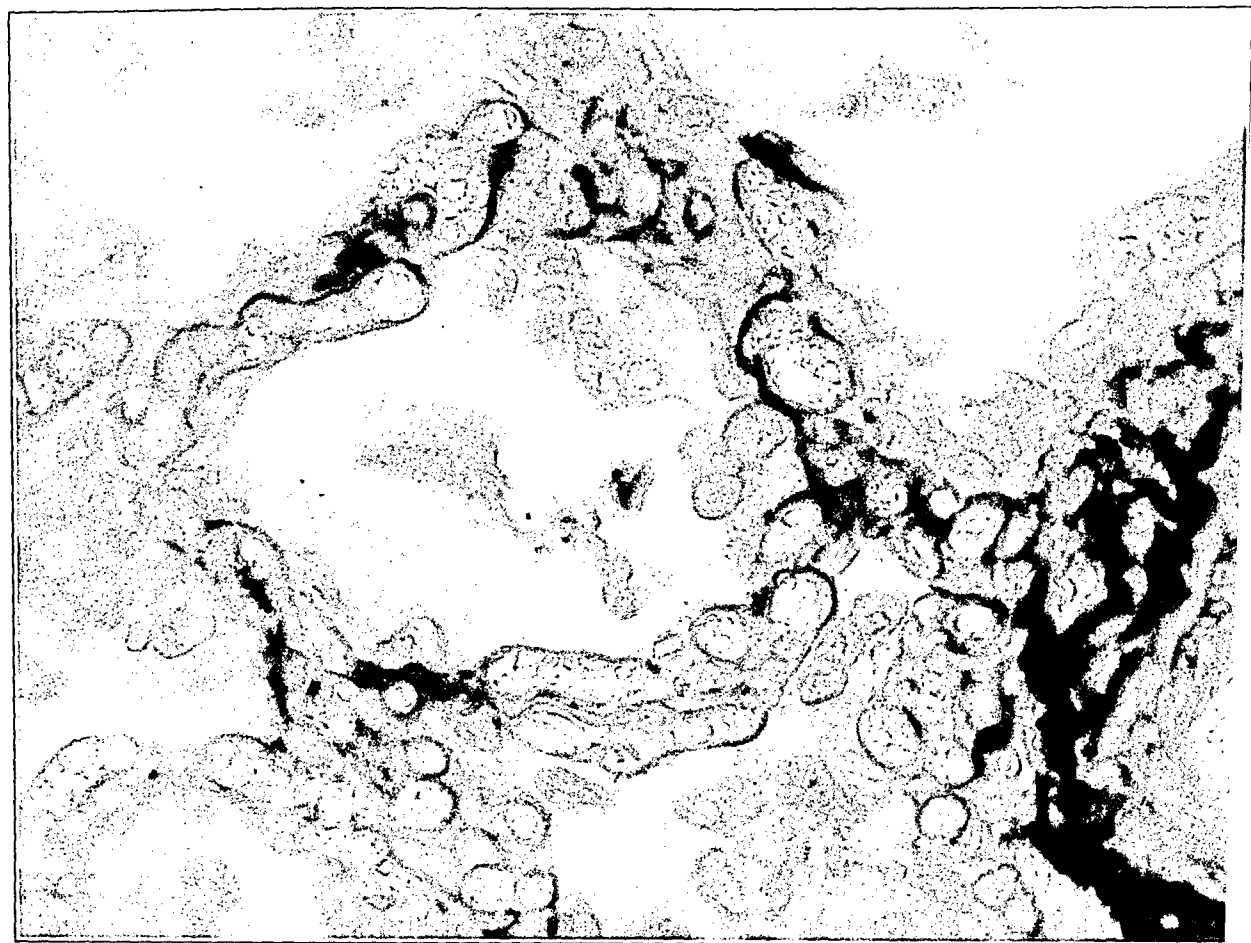
PLATE 108

FIG. 5. Section from tip of apex from Case 1. Congestion and increase in number of visible capillaries, and dilatation of same with herniation into alveolar spaces. Each capillary is sufficiently wide to admit the simultaneous passage of several red cells. Aniline blue stain.  $\times 670$ .

FIG. 6. A somewhat more advanced stage than that shown in Fig. 5. In addition to the dilatation and increased number of visible capillaries, there is thickening of the capillary basement membrane. Aniline blue stain.  $\times 600$ .



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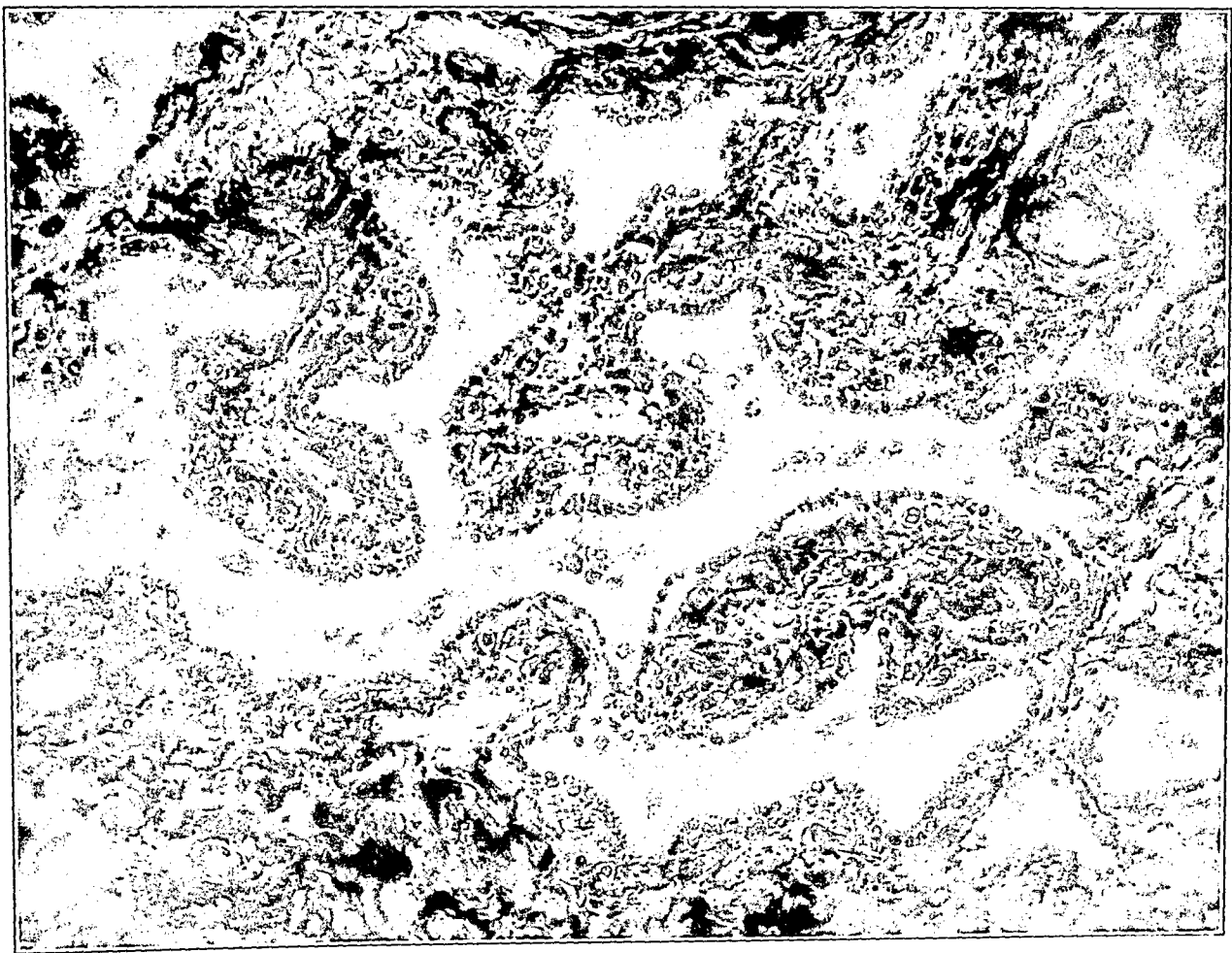


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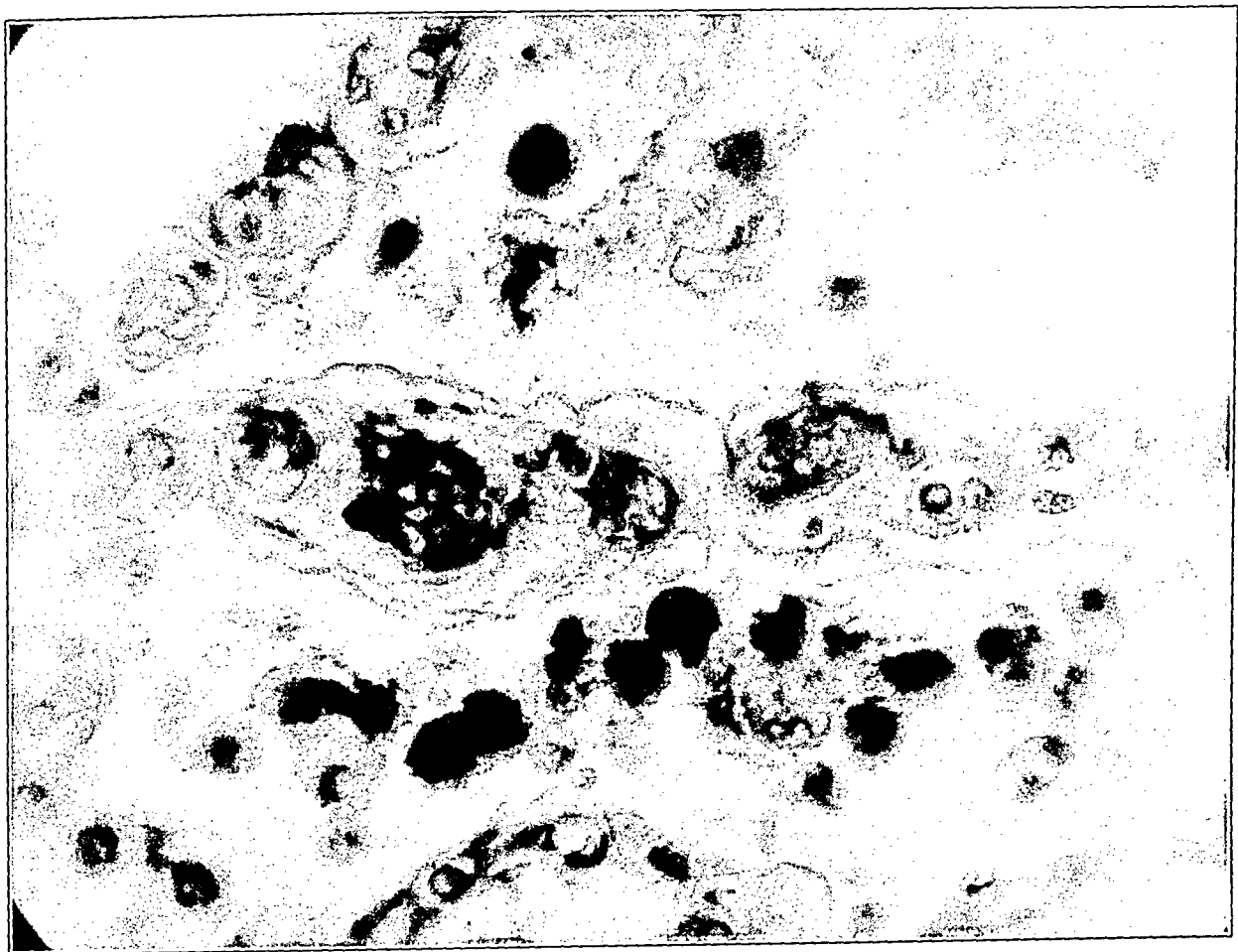
PLATE 109

FIG. 7. Late stage. Alveolar walls thickened and covered with cuboidal cells. Capillaries small and displaced from the alveolar spaces. Aniline blue stain.  $\times 210$ .

FIG. 8. Pericapillary edema. The thin, unchanged alveolar basement membrane is ballooned out by the fluid. Aniline blue stain.  $\times 670$ .



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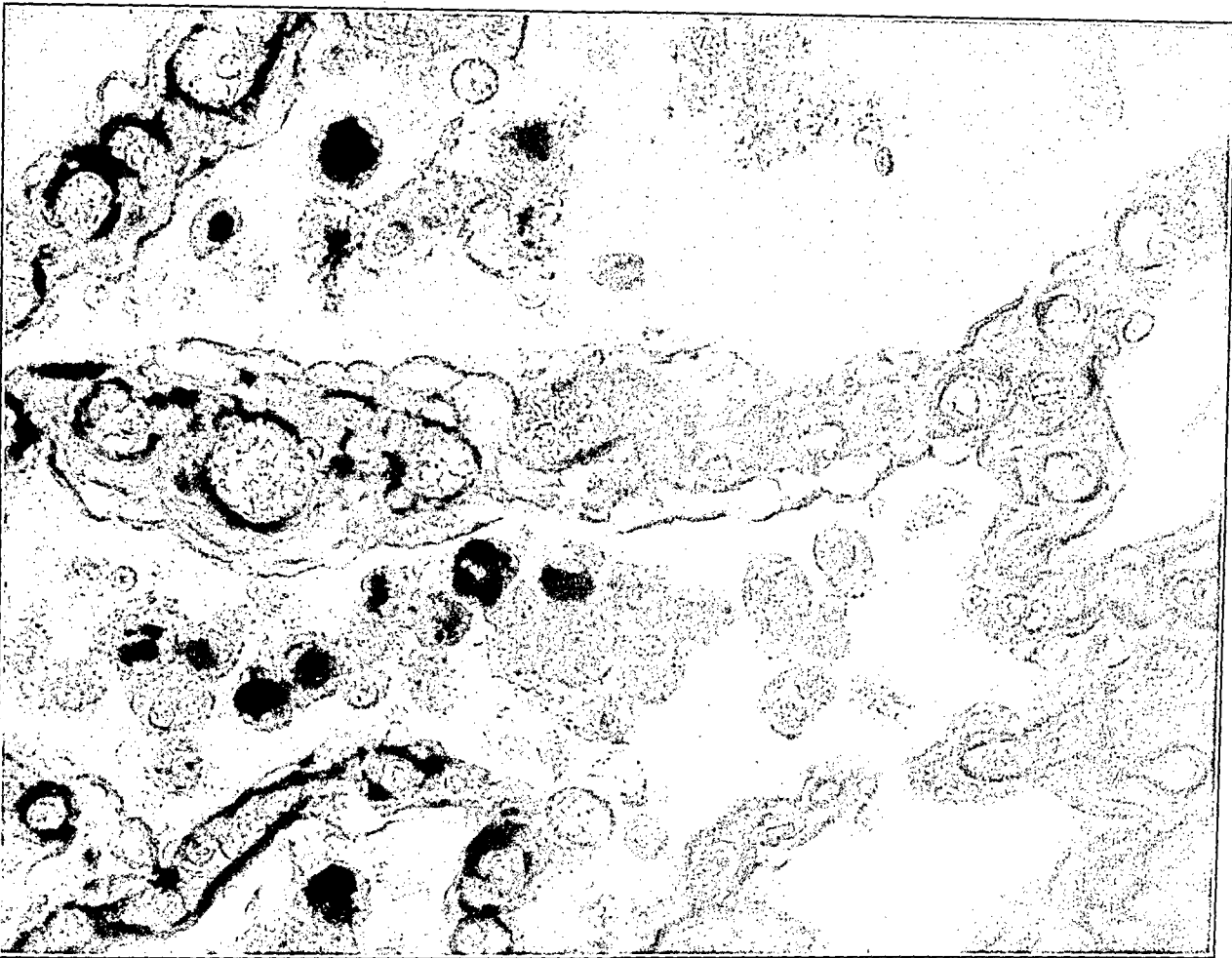


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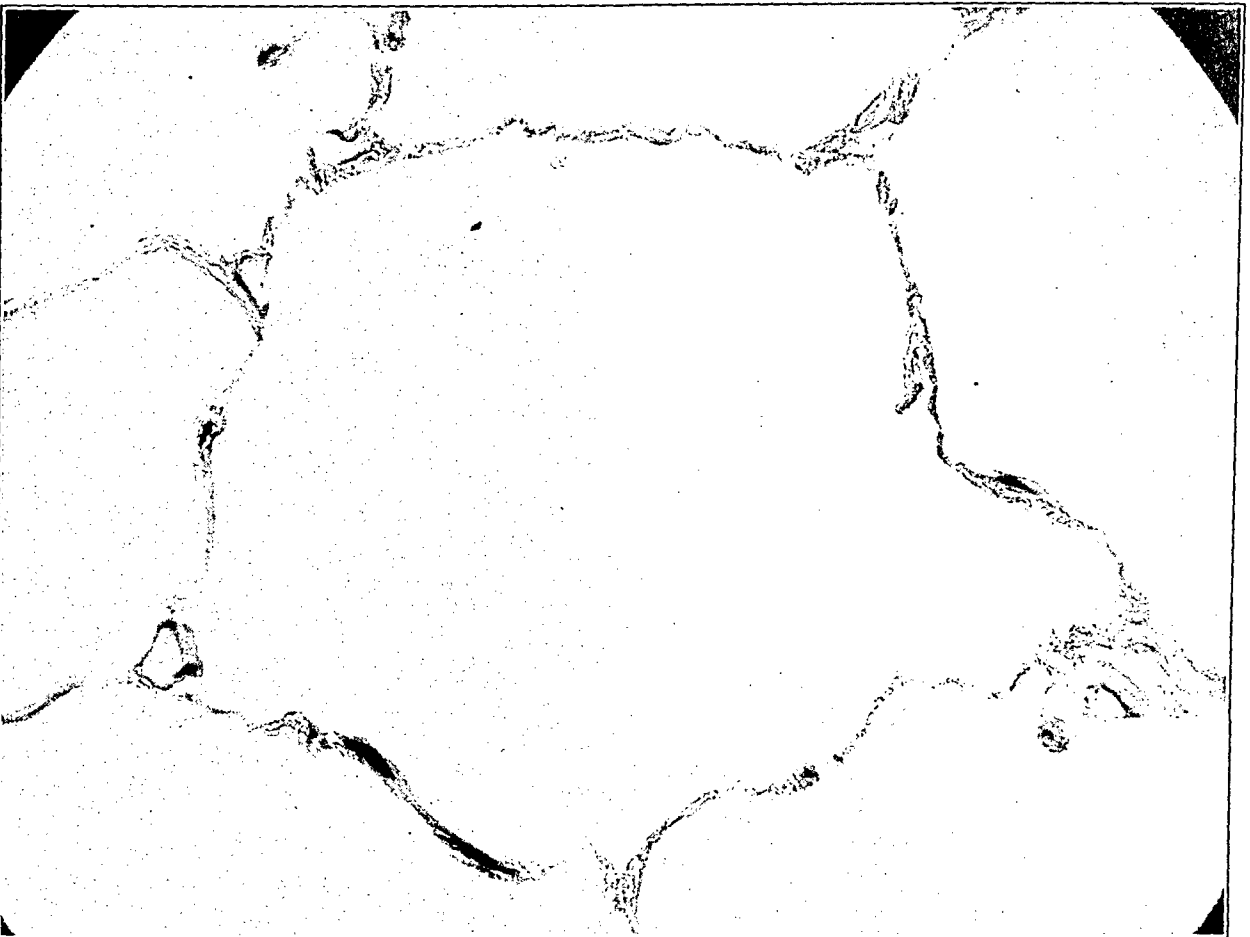
PLATE 110

FIG. 9. Pericapillary edema. Alveolar basement membrane as in Fig. 8. In addition, thickening of capillary basement membrane. Aniline blue stain.  $\times 600$ .

FIG. 10. Emphysema of a previously congested alveolus as indicated by increased collagen in the wall. Capillary lumens less than normal in diameter. Aniline blue stain.  $\times 285$ .



9



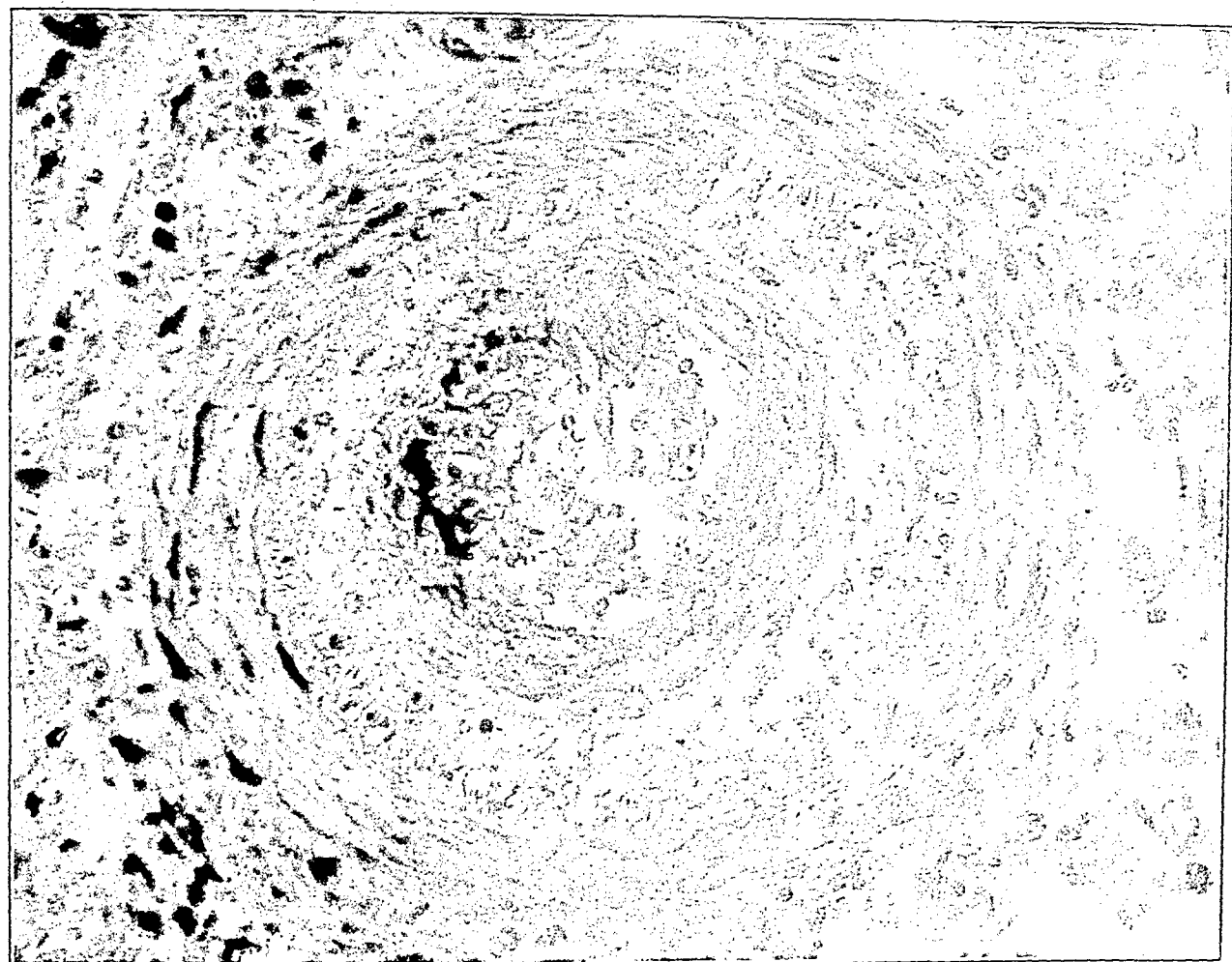
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PLATE III

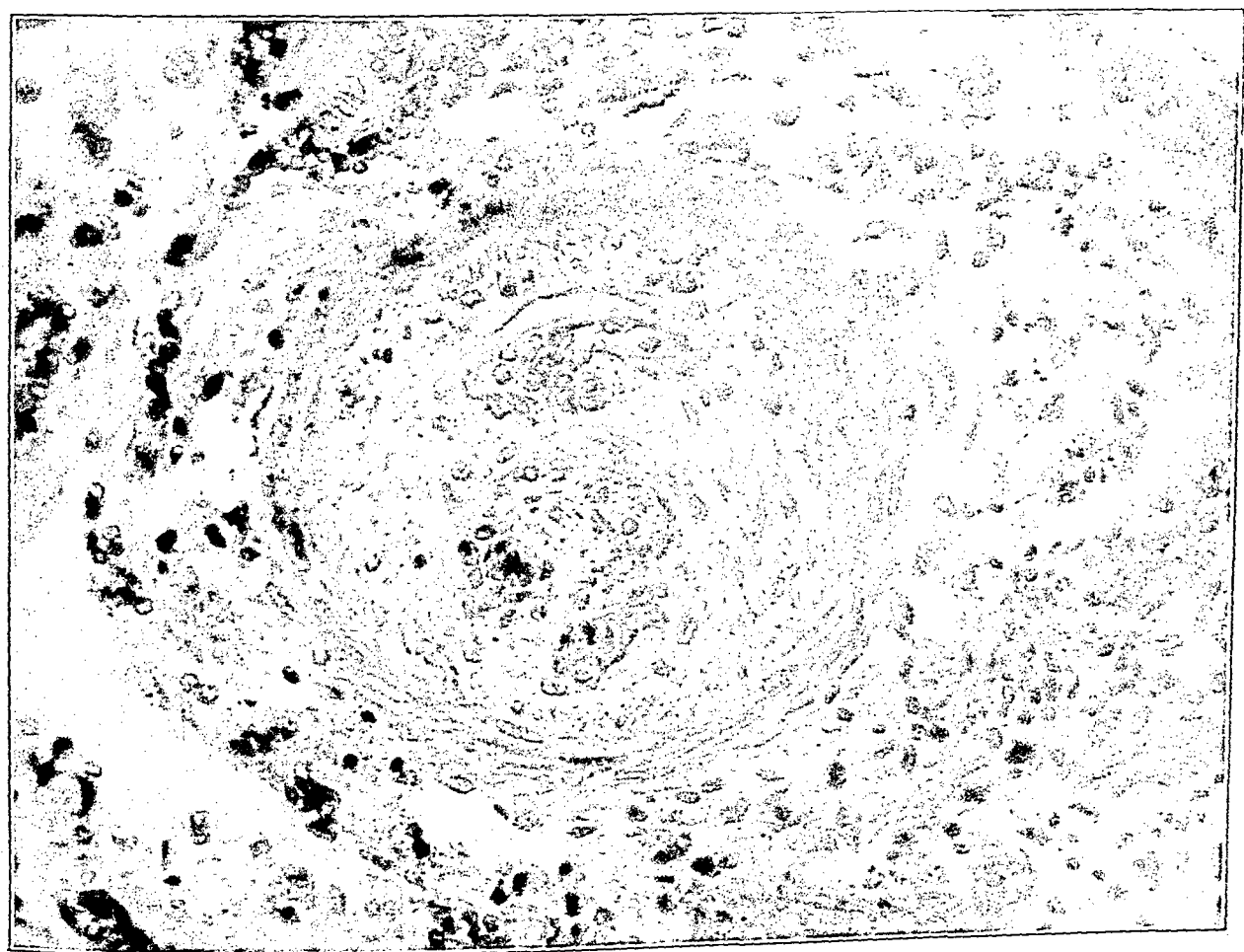
FIG. 11. Case 1. Rheumatic arteritis, early stage. Subendothelial deposition of fibrin with proliferation of endothelial cells. Phloxine-methylene blue stain.  $\times 360$ .

FIG. 12. Case 1. Rheumatic arteritis, late stage. Lumen represented by endothelial lined spaces. Intima consists of vascular connective tissue. Phloxine-methylene blue stain.  $\times 360$ .





I1

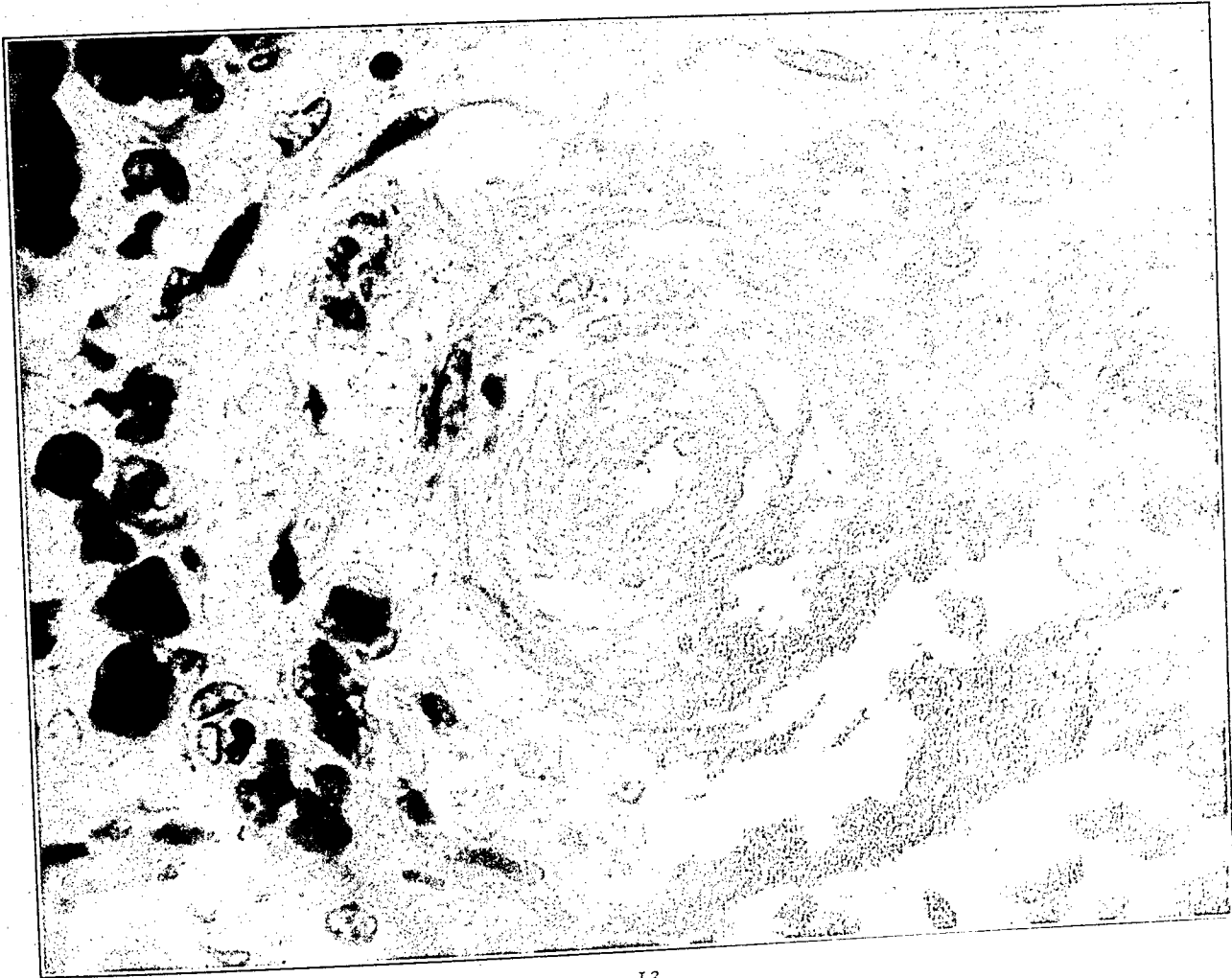


I2

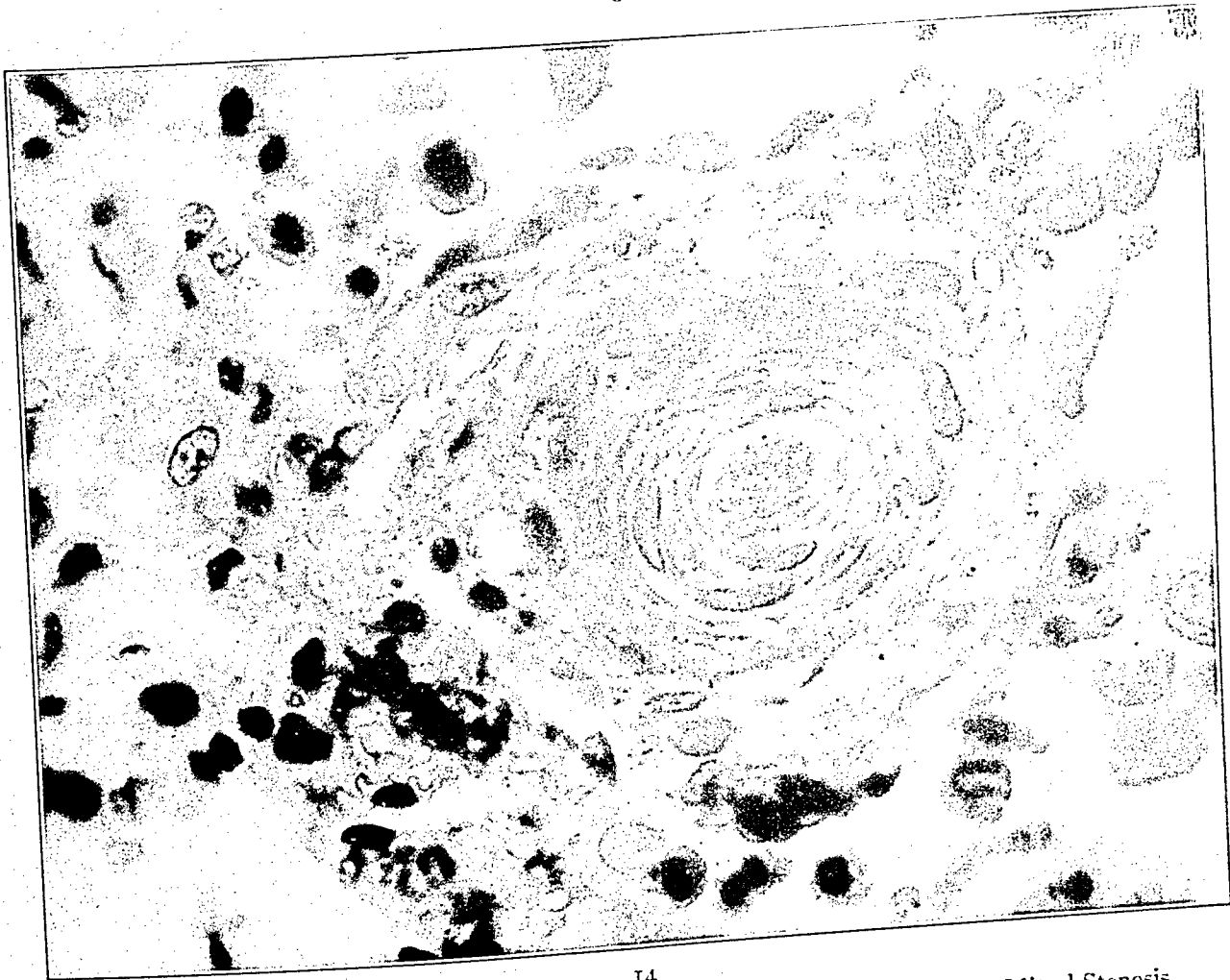
PLATE 112

FIG. 13. Hyperplastic arteriolosclerosis. Phloxine-methylene blue stain.  $\times 800$ .

FIG. 14. Hyperplastic arteriolosclerosis. Phloxine-methylene blue stain.  $\times 800$ .



13



14

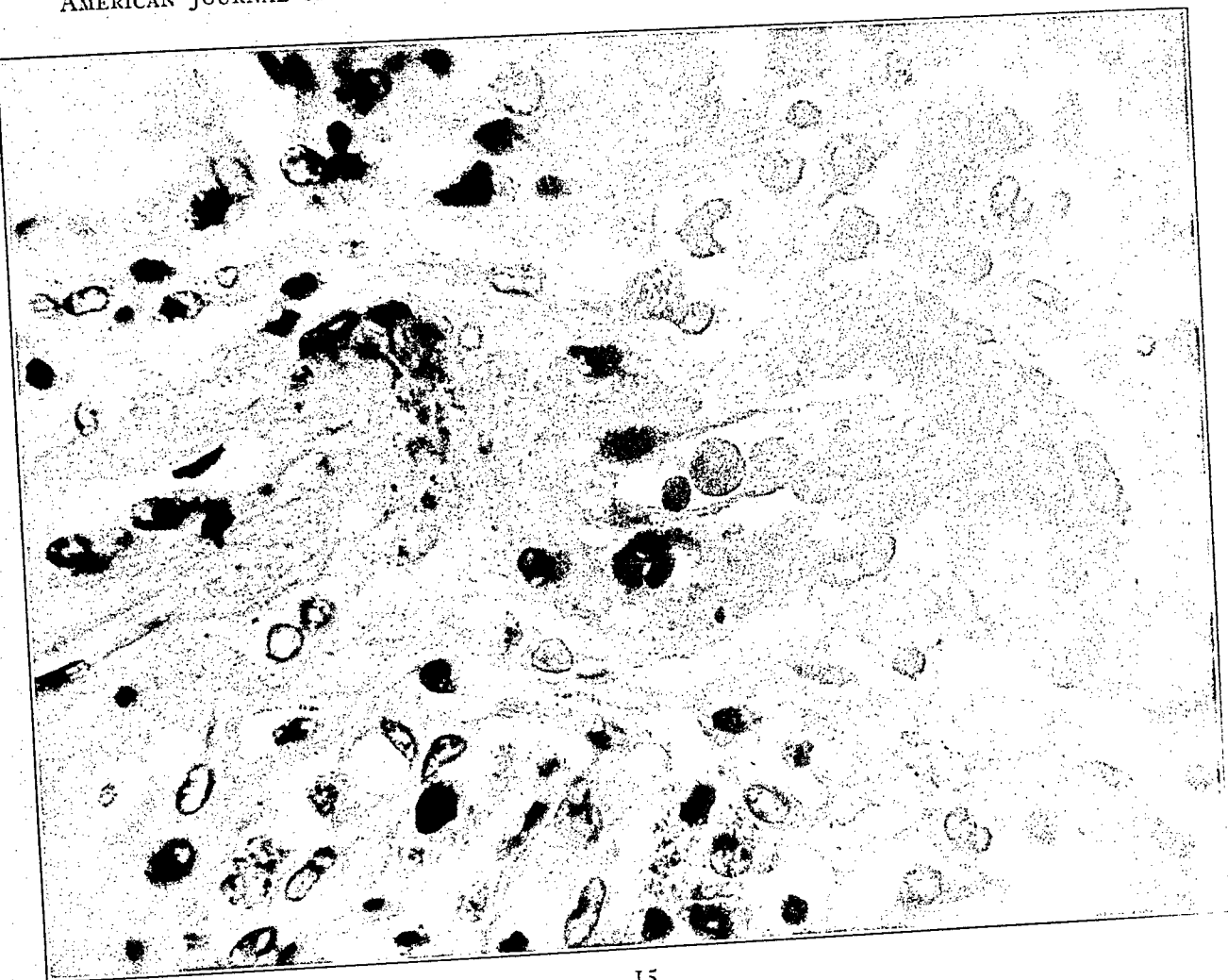
Structural Changes in Lungs in Mitral Stenosis

PLATE 113

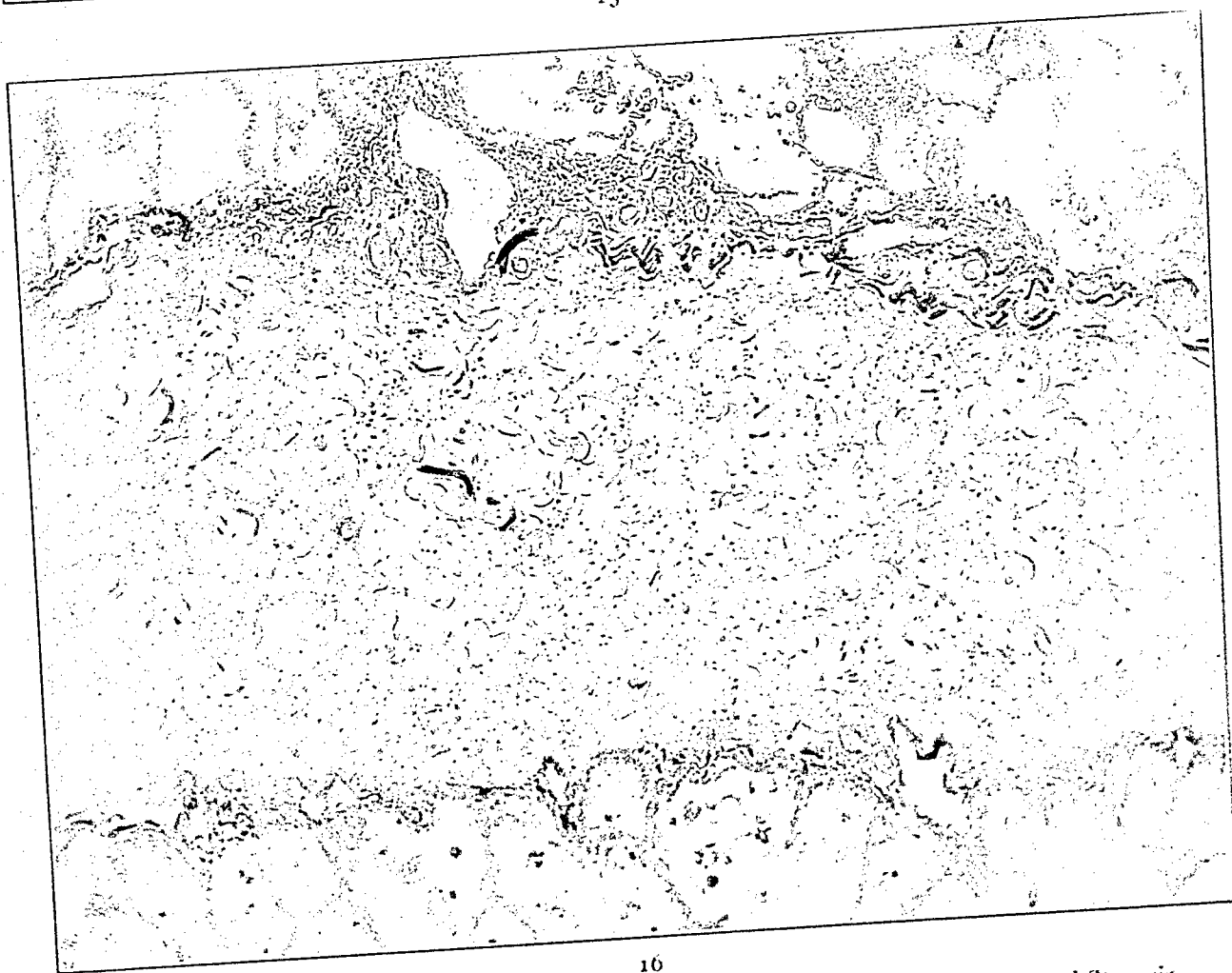
FIG. 15. Necrotizing arteriolitis. Phloxine-methylene blue stain.  $\times 800$ .

FIG. 16. Edema of septum with no edema of adjacent alveoli. Aniline blue stain.  $\times 80$ .





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# THE EVOLUTION AND INVOLUTION OF THE PROSTATE GLAND \*

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## INTRODUCTION

The earlier literature contains excellent studies on the histology of the prostate by Walker,<sup>1</sup> Langerhans,<sup>2</sup> Moullin,<sup>3</sup> Petersen,<sup>4</sup> Weski,<sup>5</sup> and Pallin.<sup>6</sup> Investigations of the structure of the prostates of animals have also been made by Walker,<sup>7</sup> Stilling,<sup>8</sup> and de Bonis.<sup>9</sup> The entire literature on this subject is reviewed by Macklin.<sup>10</sup> In addition a few investigators, particularly Englisch,<sup>11</sup> have published reports on senile atrophy of the prostate. The surgical aspects of this atrophy are well discussed by Datyner,<sup>12</sup> Dubs,<sup>13</sup> and Shen.<sup>14</sup> In none of these studies is the histological appearance correlated with the age and related prostatic and visceral disease so that etiology and pathogenesis can be determined. The recent investigations on the sex hormones, summarized by Allen and his collaborators,<sup>15</sup> have opened a new mode of approach to these problems. By experiments with partially endocrinectomized animals with controlled parenteral administration of hormones the morphological appearances in man may be reproduced and correlated.

The present study was undertaken originally at the suggestion of Prof. J. Erdheim, in Vienna, as a morphological investigation, and later the experimental aspects were undertaken at Cornell University under Dr. Eugene L. Opie. The morphological studies were carried on and largely completed at Western Reserve University under Dr. Howard T. Karsner. To these three I am indebted for their guidance and support. The National Research Council, through the Medical Fellowship Board and the Committee on Grants-in-Aid, has made possible the technical part of the work.

The basic material for study consisted of 678 prostates secured from consecutive autopsies at the Prosektur of the Krankenhaus der Stadt Wien from August 1931 to July 1932. The prostate was secured at autopsy, left uncut and fixed for 10 days to 6 months in

\* Received for publication February 17, 1936.

10 per cent formalin. After fixation the gland was sectioned sagittally with each section not over 4 mm. in thickness. Paraffin sections stained with hematoxylin and eosin were prepared from each block. The whole blocks were not subdivided unless they were too large for a 3 by 2 inch slide. Additional sections for special stains were prepared as seemed desirable. The Masson trichrome stain with light green, and the Verhoeff elastic tissue stain were especially instructive. In some instances longitudinal and horizontal sections were prepared to illustrate special points. By this step-section method of study every lesion of the prostate larger than 4 mm. in diameter, and many smaller ones, were observed. For the special study of lipids smaller blocks were separated from the whole sections, embedded in 20 per cent gelatin and frozen sections prepared. This method gives assurance that small free masses in the lumens are not lost. Routinely, these sections were stained with Ehrlich's hematoxylin and Sudan III. For special purposes Nile blue sulphate and light green and acid fuchsin were used. Every frozen section was examined with polarized light. One hundred and eighteen prostates were examined in frozen section. The incidence of the various diseases and the statistical analyses are based entirely on these 678 unselected cases. In addition, selected prostates from autopsies at the University Hospitals in Cleveland from August 1932 to July 1933, and at the New York Hospital from August 1933 to June 1935, were studied with routine single sections and a few with step-sections.

#### MORPHOLOGICAL CHANGES AT PUBERTY

In another paper,<sup>16</sup> the evolution of the prostate through the neonatal period and infancy will be presented. At about 10 years of age there is beginning activity in the prostatic epithelium and between the 12th and 14th years the richly branching alveoli with numerous papillae become evident. In Figures 1 and 2 are shown the general appearance of the prostate just before and after puberty. In a series of white rats\* of various ages the same puberal maturation has been observed, but in the rat the transition is a more gradual process and early signs of maturation of the prostatic epithelium may be seen at 40 days of age. The clear vacuole in the epithelial cell of the rat's

\* These examples have been selected from a series of 15 animals killed on the 1st, 1st, 3rd, 5th, 7th, 10th, 13th, 16th, 20th, 24th, 28th, 36th, 70th, 70th and 70th days of life. All were from the related parents and were known to be fertile at about the 60th day.



prostate after Bouin's fixation may be observed as early as the 20th day.

The explanation for this puberal prostatic maturation is part of the general problem of the cause of puberty. However, as a working hypothesis the experimental results of hormone injections may be utilized. The rapid maturation of the prostate in 48 to 96 hours, which follows the injection of either the pituitary-like hormone of pregnancy or the male sex hormone, is well known and the results of our few experiments need not be detailed or figured. However, it is clear that the histological changes in the prostate at puberty may be duplicated by the injection of either the male sex hormone or the pituitary-like hormone of pregnancy in mice or rats. This effect of pregnancy urine has been noted by others and the literature is summarized by Bourg.<sup>17</sup> Smith and Engle,<sup>18</sup> and Moore and Price,<sup>19</sup> have described similar enlargement of the accessory sex glands in immature animals after pituitary implants. That the pituitary hormones do not act directly on the prostate is shown by the results of injections in castrated rats and mice. Although hypophysectomy induces changes in the prostate identical with those produced by castration (see Smith<sup>20</sup>), the injection of pituitary extracts into castrated rats will not affect the atrophy of the prostate. From these studies it has been assumed that the pituitary hormone affects the testis, which in turn secretes a hormone that affects the prostate (see Engle<sup>21</sup>). Moore<sup>22</sup> has demonstrated that increasing amounts of testicular hormone depress the secretion of the pituitary hormone. Brouha, Hinglais and Simonnet<sup>23</sup> suggested that this response of the prostate and vesicles to pregnancy urine be used as the basis of a test to replace the Aschheim-Zondek and Friedman test in the female. On the contrary, the effects of the various pituitary extracts and implants on the process of testicular maturation are extremely confused by contradictory reports (see Engle<sup>24</sup>).

Griffiths<sup>25</sup> has described histological changes in the prostate of the hedgehog awakening from hibernation entirely analogous to those which occur at puberty. The alteration in the cellular pattern of the pituitary during hibernation, described by Cushing and Goetsch,<sup>26</sup> is probably a related phenomenon. Womack and Koch<sup>27</sup> could not demonstrate the male sex hormone in the urine of boys under 10 years of age. From the available anatomical, physiological and experimental evidence, the maturation of the prostate at puberty and

in the spring in hibernating animals is apparently the result of an increased quantity of the testicular hormone which reaches the prostatic tissues. The reported investigations at variance with this assumption indicate that there are probably other factors to be considered, such as the release of the hormone by the gland of origin, the renal threshold for the excretion of the hormone, the reactivity of the tissue to the action of the hormone, and possible interreactions with other endocrine glands, particularly the thyroid and adrenal. It is also possible that the extraction methods used by different investigators produce variations in the quantitative and qualitative yield.

#### MACROSCOPIC APPEARANCE OF THE NORMAL PROSTATE

On both macroscopic and microscopic examination it is possible to divide the structures contained within the capsule of the prostate into several groups: (1) the urethra and the small evaginations from it; (2) the deferential canal, which contains the ejaculatory ducts, prostatic utricle, and at times a part of the seminal vesicles; (3) the periurethral glands; and (4) the prostate proper.

The parts designated as prostate may be further subdivided into the stroma and epithelial-lined ducts and acini. On the basis of the location of the acini and the orifice of the ducts the lobules of the prostate are collected into lobes and designated as posterior, anterior, lateral and middle (Fig. 3). Lowsley<sup>23</sup> has shown that this lobar division has both an anatomical and an embryological basis. The posterior lobe is that group of glands posterior to the urethra and deferential canal, the ducts of which empty into the floor of the urethra caudad to the verumontanum. The anterior lobe comprehends those acini directly anterior to the urethra, the ducts of which empty on the roof of the urethra. The middle lobe is between the urethra and the deferential canal and its ducts are found in the floor of the urethra cephalad to the verumontanum. The lateral lobes are paired, located on the lateral aspect of the urethra and the ducts empty in the furrows on each side of the verumontanum. With the exception of the rare cases in which the middle lobe is absent, these lobes are a constant finding. Early in development the terminal acini become intertwined with one another and there is not a distinct line of separation in any one section.

The periurethral glands, usually divided into a subtrigonal group

and a collicular group, must be considered by the pathologist since numerous investigators have claimed that the glandular tissue in benign enlargement is derived from them and not from the prostatic acini or ducts. The structures of the deferential canal and the urethra are not directly concerned with the scope of this study and will not be discussed.

On sagittal section (Fig. 4) through the center of the gland the typical macroscopic appearance can be demonstrated. The gray or grayish yellow ducts to the lateral lobe radiate from the posterior extremities of the V or inverted Y shaped urethra. The acini appear as light orange-yellow, slightly elevated granules with or without a lobular arrangement. The posterior lobe is directly posterior to the urethra but at its lateral borders cannot be sharply delimited from the lateral lobes. The acini are usually more distinctly orange-yellow or orange in color, in contrast with the purer yellow of the other lobes, and the lumens or even slight depressions in the center of each gross acinus cannot be seen as clearly as in the lateral lobe. The anterior lobe may usually be recognized grossly. The acini are gray or grayish white in color and few in number. The lateral lobe is large and the acini definitely apparent. They are usually gray or grayish yellow but some isolated areas may be orange-yellow. Anterior and medial to the ducts the lobular markings are distinct and lumens can rarely be seen. These glandular groups are usually yellowish than the remainder. Lateral to the ducts, lobules cannot be as easily identified and dilatation of the acinar lumens is common, especially just beneath the capsule. The stroma throughout the entire gland is gray or grayish white and opaque, but in the posterior lobe it may be bluish gray and semitranslucent. About the urethra, and separating it from the lateral lobe, there is a definite zone of gray fibrillar tissue which contains only an occasional small gray acinus.

On midlongitudinal section the general appearance of the acini is the same. The posterior lobe can now be sharply delimited by the urethra, deferential canal and posterior capsule. The ducts are yellow or yellowish orange and are directed toward the urethra caudad to the verumontanum. The middle lobe is clearly seen anterior to the deferential canal and posterior to the urethra. The acini, especially in the cephalic part, are deep yellow in color. The ducts empty just cephalad to the verumontanum and are gray in color. Anterior to the middle lobe, in the floor of the urethra, the periurethral glands

can usually be seen as small gray or grayish white dots in the gray fibrillar tissue. The acini of the anterior lobe, if present, are above or just cephalad to the verumontanum in the roof of the urethra.

By exact observations of the gross structure certain deductions may be made concerning the character of the glands and the epithelium. A pure yellow color of the lobules is indicative of tall epithelium and is not related to lipid content; the deeper the yellow color in the gross, the taller the epithelium. A gray color of the lobules is almost constantly associated with a low cuboidal epithelium. In contrast to the pure yellow, the addition of an orange element means the appearance of demonstrable lipid in the epithelial cells. This lipid can be stained with scharlach R and Sudan III and a part of it is doubly refractile. As a confirmatory observation on the character of the epithelium the character of the granulation of the lobules may be employed. A coarsely granular cut surface within each lobule is evidence that the epithelium is thrown into papillary folds, while a finely granular surface is associated with small round acini with cuboidal epithelium and free of papillae. In the ducts the variation in color from gray to yellow is the result of the presence of transitional or columnar epithelium respectively. A pale yellow zone about the larger ducts is due to the concentration of elastic fibrils in this region. The color of the stroma is an admixture of the color of smooth muscle and collagenous connective tissue.

#### SIZE OF THE PROSTATE AT DIFFERENT AGES

When blocks were cut from the fixed prostates, measurements of the three axes were made in order to secure information on the size in the successive decades. After the sections were prepared, all sections of each prostate were examined for pathological changes other than those which will be discussed later as typical of senile involution. With these criteria there are available measurements on 129 prostates. It is freely acknowledged that there may be some alterations that would affect the size of the gland and that have not been recognized. All cases of benign enlargement, carcinoma and manifest infection have been eliminated, regardless of the size of the lesions. The volume has been calculated on the assumption that the prostate is a flattened prolate spheroid and that the formula

$$\text{Volume} = \frac{4}{3} \pi a b c$$

is applicable when  $a$ ,  $b$  and  $c$  are the semiaxes.

TABLE I  
*Size of Prostate*

Decade	Observations*	Volume	Cephalocaudad axis	Lateral axis	Anteroposterior axis
		cm.	cm.	cm.	cm.
3rd ....	22	10.22 $\pm$ 1.09	2.75 $\pm$ 0.116	3.60 $\pm$ 0.119	1.89 $\pm$ 0.076
4th ....	24	11.72 $\pm$ 0.799	2.92 $\pm$ 0.111	3.71 $\pm$ 0.088	1.95 $\pm$ 0.193
5th ....	18	10.88 $\pm$ 0.788	2.87 $\pm$ 0.085	3.67 $\pm$ 0.083	1.96 $\pm$ 0.109
6th ....	31	12.03 $\pm$ 0.804	2.91 $\pm$ 0.071	3.75 $\pm$ 0.086	2.06 $\pm$ 0.076
7th ....	22	12.06 $\pm$ 1.01	2.85 $\pm$ 0.114	3.68 $\pm$ 0.085	2.05 $\pm$ 0.087
8th ....	9	11.94 $\pm$ 1.11	2.87 $\pm$ 0.080	3.78 $\pm$ 0.125	2.05 $\pm$ 0.115
9th ....	3	13.70 $\pm$ 1.39	3.00 $\pm$ 0.205	4.37 $\pm$ 0.173	2.17 $\pm$ 0.238

\* The 120 individual observations have been studied by the statistical method of analysis by variance for significant differences between the decades.

TABLE II  
*Analysis of Variance of Volume of the Prostate in Different Decades*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total .....	128	2,351.00	18.36
Between decades .....	6	74.67	12.44
Within decades .....	122	2,276.33	18.65

F 1.49

TABLE III  
*Analysis of Variance of Cephalocaudad Dimension of the Prostate in Different Decades*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total .....	128	26.87	0.21
Between decades .....	6	0.50	0.08
Within decades .....	122	26.37	0.22

F 2.75

TABLE IV  
*Analysis of Variance of Lateral Dimension of the Prostate in Different Decades*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total .....	128	26.14	0.20
Between decades .....	6	1.66	0.28
Within decades .....	122	24.48	0.20

F 1.40

TABLE V

*Analysis of Variance of Anteroposterior Dimension of the Prostate in Different Decades*

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total .....	128	29.17	0.23
Between decades .....	6	0.59	0.10
Within decades .....	122	28.58	0.23

F 2.30

TABLE VI

Case No.	Estimated age	Actual age	Difference	Case No.	Estimated age	Actual age	Difference
	yrs.	yrs.	yrs.		yrs.	yrs.	yrs.
<i>Syphilis</i>				<i>Diabetes</i>			
328	65	62	+ 3	177	58	71	-13
353	60	62	- 2	240	55	66	-11
354	63	51	+12	294	60	59	+ 1
401	53	57	- 4	321	65	52	+13
408	75	72	+ 3	347	63	67	- 4
446	65	68	- 3	511	53	57	- 4
466	68	70	- 2	529	62	57	+ 5
478	70	73	- 3	<i>Leukemia</i>			
482	55	71	-16	378	70	65	+ 5
493	62	74	-12	418	60	74	-14
498	55	54	+ 1	257	30	39	- 9
546	60	54	+ 6	<i>Chronic Nephritis</i>			
563	60	58	+ 2	405	55	63	- 8
575	62	69	- 7	277	50	55	- 5
597	65	61	+ 4	93	25	18	+ 7
<i>Rheumatic Heart Disease</i>				<i>Hypertension</i>			
125	40	40	0	277	57	55	+ 2
131	60	65	- 5	332	60	58	+ 2
222	65	74	- 9	360	60	66	- 6
232	65	68	- 3	489	65	70	- 5
272	70	63	+ 7	493	70	74	- 4
302	62	74	-12	496	62	62	0
376	35	43	- 8	501	65	66	- 1
387	40	32	+ 8	513	68	63	+ 5
416	60	60	0	<i>Carcinoma</i>			
<i>Streptococcic Infections</i>				264	57	59	- 2
377	50	55	- 5	266	72	67	+ 5
479	60	58	+ 2	273	62	54	+ 8
563	65	58	+ 7	286	60	50	+10
<i>Multiple Sclerosis</i>				292	72	70	+ 2
272	68	63	+ 5	297	70	70	0
296	60	50	+10	335	40	45	- 5
361	35	24	+11	342	70	71	- 1
391	55	39	+16	362	60	64	- 4
512	58	62	- 4				

In these analyses of variance,  $F$  is equal to the greater mean square divided by the smaller mean square and when the observations of the former are infinite and of the latter 7 the least significant  $F$  is 3.23. In the reverse, the  $F$  must exceed 2.17 to be considered as showing a greater difference between the decades than within the decades. Therefore, we may conclude that on the basis of 129 observations there is no significant difference in the size of the prostate between 20 and 90 years of age.

### MICROSCOPIC APPEARANCE OF THE POSTPUBERAL PROSTATE

Microscopic examination of a midsagittal section similar to that described above reveals the picture shown in Figure 5. This section is taken just cephalad to the orifice of the ejaculatory ducts so that acini of the middle, posterior and lateral lobes are shown. The middle lobe acini anterior and immediately lateral to the deferential canal are sharply separated from the lateral and posterior lobe by stromal septa. The septal markings between the posterior and lateral lobes are not definite. The general lobular architecture is well shown in all parts of the gland and even with this magnification the rich papillary folding of the epithelium is evident and the papillae are equally abundant in all parts, a point that is important as a criterion of adult life. The lobular architecture both grossly and microscopically should be sharply differentiated from the nodular architecture of benign enlargement.

The epithelial cells of the prostate during the 3rd and 4th decades vary considerably in size and structure. In this study an attempt has been made to classify these cells and arrange them in a hypothetical cycle of secretion.

The first type (Fig. 6) is a low columnar or cuboidal cell with a round, moderately chromatic nucleus situated in the base of the cell but not in contact with the cell wall. The nucleus may be of such a size as to occupy two-thirds of the cell, although it usually fills about one-half. The cytoplasm, with formalin or Zenker fixation, is palely acidophilic, slightly reticulated and free from granules. Beneath the cuboidal cell there is an irregular layer of basal cells that can be clearly distinguished as distinct from the surrounding stroma. The nuclei of these basal cells are elliptical with the long axis in the plane of the wall of the acinus. The cytoplasm is inconspicuous but in gen-

eral is slightly denser than that of the luminal cells. The luminal cells vary from 8 to 12 microns in width and each cell is a distinct entity with no pseudostratification of cells or nuclei. The luminal cell wall is well defined and may be either smooth or slightly scalloped on account of bulging of the cytoplasm of each cell. When viewed from the lumen such a group of cells as described would present slight mammillation. This cell varies from 10 to 15 microns in height.

The second type (Fig. 7) is a much taller cell with a slightly smaller, round nucleus. The cytoplasm is essentially the same as that of Type 1 but the cell is only 7 to 10 microns in width. In height it varies from 15 to 20 microns and the cell walls including those toward the lumen are distinct. The basal cells are generally similar to those of Type 1.

The third type presents radical differences from those above described (Fig. 8). The cell is from 18 to 25 microns in height and 9 to 12 microns in width. The basal and lateral cell walls are distinct but the luminal membrane is indistinct or may be entirely lacking, so that it appears that the cytoplasm of the cell is directly continuous with secretion within the lumen (Fig. 9). When there is a remnant of the luminal cell wall the nucleus is elliptical in shape with the long axis parallel to the wall of the acinus, but in those cells where there is rupture the nucleus is round or at the most only slightly ovoid. When the nucleus is elliptical a basal layer of cells cannot be demonstrated but if the nuclei are round the basal cells are as distinct as in Types 1 and 2. The cytoplasm is considerably denser and more acidophilic than that of the earlier described types and when the luminal membrane is intact the cytoplasm is clearly denser in a zone of 1 to 3 microns beneath it. In this denser luminal cytoplasm and extending down into the clearer cytoplasm there are small, variously sized granules which are for the most part acidophilic, but an occasional one is basophilic. The lumens bounded by the epithelium of this type invariably contain granular acidophilic debris which we have considered as prostatic secretion.

The fourth type (Fig. 10) is materially different from any of the previous three types. The nuclei are elliptical with the long axis perpendicular to the wall of the acinus. The chromatin is much denser and basophilic and an internal chromatin network can be demonstrated only with difficulty. The nuclei are closely opposed to



one another with pseudostratification. The cytoplasm is densely homogeneous and more acidophilic than that of the other types. Cell walls are not apparent but the luminal boundary of the cell is smooth and distinct.

The only remaining cell type of the prostatic acini and ducts is the transitional epithelium which is frequently found in the major ducts near the urethra. It conforms entirely to this type of epithelium described in textbooks and need not be considered in detail here.

These various types of epithelial cells and cells that appear to be transitional stages between them may be found in one prostate, but in general there is a tendency for one type to be present in the greater number of acini. Within one acinus two or more types may be found, but again the greater number of cells are of one type.

These cell types are found on the walls of the acini and over the papillae although what is apparently the same cell type is taller and thinner on the sides and tips of the papillae than on the wall. The nuclei are in general more chromatic near the tips of the papillae.

Many of the papillae of the acini in a prostate from an adult male, when studied in serial section, are apparently a phenomenon of local growth. With the increase in the cytoplasmic content of each cell some of the increased pressure is released toward the lumen and manifested as an increase in height of the cell and a bulging of the luminal cell wall, but at some point the pressure is greater and the cells buckle into a small mound without a stromal core. The nuclei in these areas are elliptical with the long axis at right angles to the wall and each cell is very narrow. Other, usually larger, papillae contain a connective tissue centrum with an occasional smooth muscle fiber.

The stroma of the prostate is composed of three essential elements — collagenous connective tissue, smooth muscle fibers and elastic fibers. The arrangement of these elements about the individual acini and the connections with the musculature of the bladder and ejaculatory canal have been studied by Walker,<sup>1</sup> Lowsley,<sup>28</sup> and others, and the possible relation of this anatomical structure to the physiology of urination and ejaculation adequately discussed.

The stroma may be divided into classes, dependent on the location, into periductal, interlobular, intralobular or interacinar, perivascular and perineurial types.

About the larger ducts there are bundles of smooth muscle fibers

which are arranged both longitudinally and circularly or spirally. The former are fine fibers and are arranged in small bundles while the latter are gathered together in larger bundles. Between the muscle fibers in both instances there is a moderate amount of collagenous connective tissue and elastic fibers. The longitudinal elastic fibers are more prominent.

About all the larger and medium sized vessels and nerves there is a layer of pure fibro-elastic connective tissue without smooth muscle fibers. The vessels of arteriolar size and smaller ramify directly in the interlobular and interacinar stroma.

The stroma between and in the lobules is essentially the same although the muscle fibers are more abundant and somewhat larger in the interlobular septa. About the acini there is a definite disposition of the stroma which is of the greatest importance in the proper diagnosis of pathological changes. Immediately external to the cell wall of the epithelial cells there is a thin zone of pure collagenous connective tissue and a few elastic fibrils. In this layer are long, narrow, at times wrinkled nuclei — presumably fibrocytic nuclei. A few of the capillaries are found between these collagenous fibrils but an endothelial lined channel has never been observed in direct contact with epithelium. Outside this layer is stroma composed of approximately equal parts of smooth muscle and connective tissue arranged in sweeping arcs about the acini in such a manner that contraction of the muscle would result in reduction of the size of the lumen from all sides equally.

#### MORPHOLOGY OF THE PROCESS OF PROSTATIC SECRETION

The difficulties of a study of a secretion process, on the basis of isolated morphological observations on autopsy specimens, are apparent. In the evidence which has been presented it would appear that there is a cycle in the epithelial cells. The small cuboidal cell is the type of cell that is designated as a "resting cell" in other glands where secretion has been studied. In contrast is the tall columnar cell of Type 3 with the cytoplasm continuous with secretion in the lumen, a process of apocrine\* secretion that has been described in other glands. Type 4 is definitely a cell of hyperplasia with crowding

\* The term apocrine is used here to designate a process in which a portion of the cytoplasm of a cell is discharged as secretion. When the entire cell is converted into secretion the term holocrine is applied to it.

of the nuclei and an increase in the intensity of staining. In order to secure a working hypothesis the cell types have been ranged in a drawing (Fig. 11) in the order these observations indicate is correct. There is no related change in the stroma as the cells increase in height and discharge a portion of their cytoplasm.

### MORPHOLOGY OF THE PRESENILE PROSTATE

Manifestly, any classification of a gradual process is artificial and therefore no attempt will be made to describe the morphology of the prostate during each decade after 40 years. However, it would appear that the involution proceeds with greater rapidity during the 5th and 6th decades and that after 60 years the changes are less striking and the velocity is greatly decreased. Therefore, the process has been divided into a presenile period and a senile period with the dividing line at approximately the 60th year of life. In any one individual or when only one morphological criterion is employed, the age of onset of definite senility will vary, but on the whole, old age, as determined by the morphology of the prostate, may be considered as definite at 60 years.

The outstanding characteristic of the presenile gland is the variation in the appearance of the same structure in different parts of the same prostate, in contrast with the uniform appearance of the adult prostate.

In the stroma there is a gradual atrophy of the smooth muscle fibers and relative or perhaps absolute increase in connective tissue. The cells of the connective tissue retain their adult characteristics and there is no histological evidence of proliferation. The nuclei are small, highly chromatic and with increasing age may show irregularity in outline and even pyknosis. The collagen fibrils become denser and agglutinated, although clear hyalinization is rare in the presenile period. There is an increase of the collagenous tissue about each acinus so that the layer, which in adult life is 5 to 15 microns in thickness, is expanded to a layer 20 to 50 microns. The smooth muscle fibers are smaller but pigmentation is not a consistent part of the picture. When pigmentation of these fibers is found the granules are similar to those found in other organs with atrophy and senility.

The acini at this period are in general larger and the papillae are not as numerous, but a not inconsiderable number of alveoli are

lined by an epithelial layer essentially the same as in the earlier decades. The hyperplastic cells described as Type 4 are more conspicuous and pseudostratification is more apparent (Fig. 10). The nuclei are more chromatic and the cytoplasm more acidophilic and denser, that is, the morphological criteria of hyperplastic cells. The majority of papillae that are present are of the same two types described in the adult prostate, but in addition there is another type observed at this period. The cells are thrown into a pedunculated spherical mass without a connective tissue centrum and in the center of such a mass there is not infrequently a lumen. Thus, there is formed an acinus with a lumen within the epithelium of the wall of a larger acinus. The individual epithelial cells in these pseudo-acini are deeply acidophilic and denser and the nuclei are larger and more chromatic than the cells of the adjacent wall. A nucleolus is not conspicuous and the shape of the cell varies from polygonal to columnar with or without orientation as regards the major or minor acinus.

Metaplasia may occur during the presenile period. There is a focal area, usually in the ducts but occasionally in the terminal alveoli, where the usual epithelium is replaced by a mass of spindle cells with general orientation of the long axis at right angles to the wall of acinus. The luminal cells are more cuboidal and definitely oriented toward the lumen. A basal layer or even a basement membrane is lacking but there is no evidence of invasion of the stroma. This is a solitary alteration without associated change in the stroma and without surrounding inflammatory infiltration.

In contrast with these manifestations of cellular growth, namely hyperplasia, pseudo-acinar formation and metaplasia, atrophy is a conspicuous feature of the presenile period. This atrophy may involve only the epithelial cells or the acinus as a complete structure, or the two types may be combined.

Atrophy of the epithelium results in a decrease in the height of the cell and all stages may be observed. As landmarks two types have been selected and designated as Types 5 and 6. In Type 5 (Fig. 12) the cells are in one layer and vary from 8 to 11 microns in height and 10 to 13 microns in width. The cytoplasm is granular or reticulated and a round nucleus fills at least one-half of the cell. The cell walls are distinct and the membrane toward the lumen is smooth and without the scallops seen in earlier decades in Type 1. There is no evidence of secretion.

The cell that has been designated as Type 6 (Fig. 13) is still flatter and rarely exceeds 8 microns in height. The nuclei are round or slightly flattened and are equal in diameter to the height of the cell. In a section 7 to 10 microns in thickness the nuclei overlap one another but are not stratified. The cytoplasm is homogeneous and acidophilic. The luminal cell membranes are sharp and smooth and without evidence of secretion. Rarely the nucleus may be larger than the cell so that it bulges into the lumen or into the surrounding stroma. This cellular type is the highest grade of simple epithelial atrophy that has been observed at any age and it is not seen as frequently in the presenile as in the senile period.

Associated with the atrophy of the individual epithelial cells are changes in the size and shape of the acini and in the surrounding stroma. These changes are of two types which have been designated as simple acinar atrophy and sclerotic atrophy.

Simple acinar atrophy (Fig. 14) usually involves an entire lobule, although isolated acini may be affected. The acini are small, closely packed together and lined by epithelial cells of Type 5 or 6, but some acini or individual cells may be taller and show evidence of secretion. Many of the lumens contain a granular acidophilic material similar to secretion. The surrounding stroma shows no fibrosis and the muscle fibers are abundant and whorl about the acini in the normal manner. The collagenous basement membrane is only slightly thickened.

Sclerotic atrophy is a much more complex process. The earliest lesions would appear to be a simultaneous atrophy of the epithelium and a proliferation of the fibroblasts immediately about the acinus. This proliferation may be regular so that the lumen is compressed equally and appears in section as a round or slit-like structure. It may, however, be irregular and result in distortion of the lumen (Fig. 15). Continued proliferation results in hyalinization of the collagen which is thrown in folds that follow the configuration of the original acinus. The epithelium becomes extremely flattened (Fig. 16) and eventually the sides meet and the epithelium can no longer be identified. The most centrally placed fibroblasts of the collagenous collar are loose and mesenchymal-like so that when the epithelium is lost the center of the former acinus is occupied by a loose tissue of stellate and fusiform cells without collagen (Fig. 17). With continued maturation of the central fibroblasts the whole is

converted into a small hyaline mass which is recognized with difficulty and probably eventually becomes assimilated into the stroma and cannot be demonstrated. There is at no time any exudative inflammatory reaction. Sclerotic atrophy may involve large areas, usually just beneath the capsule and in the posterior lobe, or single ducts or acini may show the lesion. The changes appear to be similar to those described by von Recklinghausen<sup>29</sup> in Bartholin's gland as *myxangioitis hyalinosa*.

The etiological factor in the production of sclerotic atrophy is not clear. It is frequently associated with arteriolar sclerosis and it is possible that the change is analogous to the atrophy and fibrosis that occur in the heart, kidney and other organs as the result of vascular disease. Since this vascular thickening is not associated with generalized arteriosclerotic disease and hypertension, it must be concluded that it represents an involutionary sclerosis similar to that which occurs in the uterus after the menopause.

### MORPHOLOGY OF THE SENILE PROSTATE

In contrast with the presenile prostate with irregularity of structure and gradual progression of changes the senile prostate represents a more static picture or at the most a very slow progression. There are no additional microscopic features which need be described. The atrophy of the epithelial cells and sclerotic atrophy of the acini become more marked so that at 80 years one-half of the acini are usually obliterated and all of the remaining cells are low cuboidal or flat and there is no evidence of secretion in any alveoli.

A midsagittal section of the prostate in the senile period shows clear differences from that of the young adult described in the previous paragraph. The cut surface is composed largely of stroma with discrete white or gray, rarely yellow granules in which there are many light brown or black corpora amylacea. The stroma in many places is pale blue and translucent. There are few dilated acini and very little fluid can be expressed from the surface. There may be focal areas of orange-yellow or orange acini which contain quantities of doubly refractile lipid.

The only remaining feature of the senile prostate is the presence of corpora amylacea, but since a separate paper<sup>30</sup> has been devoted to them it is not desirable to enter into the subject at this time.

## ESTIMATION OF AGE

On the basis of the morphological observations, which have been recorded above, it should be possible to estimate the age of an individual by a microscopic examination of a section of the prostate. This has been done on 62 prostates (Table VI) and the errors are shown in Table VII.

TABLE VII

*Estimate of Age on Basis of Morphology*

Amount of error	Age less than 74 years	Age above 75 years
0 to 5 yrs.....	58.9 %	11.1 %
6 to 10 yrs. ....	26.0 %	11.1 %
11 to 15 yrs. ....	12.3 %	38.9 %
16 to 20 yrs. ....	2.8 %	27.8 %
Above 21 yrs. ....	0.0 %	11.1 %
	84.9 %	22.2 %
	15.1 %	77.8 %

The zero order correlation coefficient of estimated and actual age is  $+0.61 \pm 0.07$ . It is apparent that the accuracy is high if the patient is less than 74 years old and that increasing age above this point results in increasing error. It has been assumed from this that the involutionary process reaches a maximum point at about this age and does not proceed further. That the morphological changes are sufficiently constant to allow of an estimate of this accuracy furnishes strong support to the conception of physiological senile involution. If the process were some pathological change or definite disease the correlation of estimated and actual age would not be so close. When an alteration of structure proceeds at a predictable rate in 85 per cent of individuals it cannot be called a disease but must be regarded as physiological and inevitable. Table VI also demonstrates that the diseases shown have no material effect on the process of involution.

The criteria on which these estimates of age have been made may be summarized as follows:

(a) Slight irregularity in the height of the epithelium begins between 40 and 45 years.

(b) Lobular atrophy begins between 45 and 50 years.

(c) The glandular epithelium loses its secretory activity between 50 and 60 years.

(d) Sclerotic atrophy first appears between 60 and 65 years.

(e) Atrophy of smooth muscle and relative or absolute increase of the fibrous tissue of the stroma is first apparent between 60 and 70 years.

(f) Laminated corpora amylacea increase in number and size after 65 years.

Thus, on the basis of microscopic structure of the prostate the life of man may be divided into several periods: first, fetal period, from the first appearance of the prostate at about the 3rd fetal month to a few weeks before birth; second, neonatal period, an interval of 2 to 3 weeks just before and after birth at term; third, childhood period, from 2 to 3 weeks after birth to 10 years; fourth, a prepuberal period, from 10 to 13 years; fifth, an adult period, from 13 years to about 45 years; sixth, a presenile period, from 45 to about 55 years; seventh, a senile period, from 55 years to 75 years; and eighth, a completely senile period, after 75 years.

As would be expected in any biological organism, every example does not fulfill these criteria. In Table VI of the estimated and actual ages of 91 prostates there is a deviation of greater than 11 years in 15 per cent of cases when the age is less than 70 years. These deviations may be divided into three classes: (1) those in which the age was overestimated under the term pathological involution; (2) those in which the age was underestimated under the term delayed senility; and (3) a group of cases in which the age may be correctly estimated but the appearance is not that which has been described for the actual age. The morphological picture of the latter is one of tall columnar epithelium in acini which show all the other characteristics of atrophy. This condition has been designated as secondary hyperplasia and may be diffuse or focal in character.

#### PATHOLOGICAL INVOLUTION

As in the previous morphological observations of senile involution, the changes of pathological involution may be divided into three groups: (1) those in the epithelium, (2) those in the stroma, and (3) the deposition of corpora amylacea. The epithelial alterations consist of a decrease in height and width of the cell, and a change in the character of the nucleus and cytoplasm. The cell, as contrasted with the finely reticulated tall columnar normal type, is low colum-



nar or cuboidal with a dense, usually acidophilic, rarely lightly basophilic cytoplasm. The nucleus is smaller and chromatic with little evidence of a chromatin thread and occupies the greater part of the cell (Fig. 18). In the usual paraffin sections there is frequently overlapping of the nuclei because the decreased diameter results in a section more than one cell in thickness. The luminal cell border is sharp and there is no evidence of secretion. The lumens are empty or contain a few partially degenerated desquamated cells. With Sudan III or polarized light there is no lipid present within the cells or lumens. The acinar atrophy has never been observed to go on to sclerotic atrophy and complete loss of acini as in the senile type.

As contrasted with senile involution the changes in the stroma are inconspicuous. This is probably because of the average short duration of the involutionary process. In the former the epithelial changes appear during the 5th decade while stromal alterations are first manifest early in the 7th decade, a difference of 15 to 20 years. Thus it may be assumed that those systemic lesions that cause involution of the adult prostate do not last long enough to bring about stromal fibrosis. At the most there is a slight relative increase of the connective tissue and decrease of the smooth muscle.

Corpora amylacea are characteristic of the involuting and involuted gland. Small corpora are found in about 25 per cent of glands from individuals between 20 and 40 years of age (in this series 25 per cent between 21 and 30 years and 21 per cent between 31 and 40 years). These corpora are associated with some atrophy of the epithelium and are usually from cases of tuberculosis or other chronic infections. In all these cases the corpora differ in general configuration from those in senile prostates. There are few concentric rings, facets are rare, central pigmentation is unusual and calcification has not been observed. Central softening occurs more frequently than in the senile gland. Many corpora are homogeneous or finely granular without internal architecture. These findings indicate that the typical corpus amylaceum in the senile prostate is the product of several months or years of growth and that the concentric lines are layers of successive deposits. In the young man with pathological involution there has been only a short period of deposition without intervening periods of non-deposition. When concentric lines occur they are less distinct, further apart, frequently wavy, and the layers are less compact. These characteristics are evidence of softer con-

sistence and less secondary organization and dehydration — again criteria of recent origin.

Thus, the histological appearance of pathological involution may be distinguished from that of senile involution on qualitative grounds. The epithelial cells undergo a similar change but complete sclerotic atrophy does not occur and the stromal fibrosis is most inconspicuous. Furthermore, the corpora amylacea exhibit differences in architecture interpreted as evidence of recent origin.

### DELAYED SENILITY

In this category have been included not only any case in which the age was more than 45 years and which showed a completely preserved adult prostate (in all sections), but also any case where the age was over 55 years and where an objective estimate of age placed it at more than 10 years less than the actual age. The incidence of delayed senility and secondary hyperplasia is shown in Table VIII.

TABLE VIII

*Incidence of Delayed Senility and Secondary Hyperplasia*

Decade	Total cases	Delayed senility		Secondary hyperplasia	
		Cases	Per cent	Cases	Per cent
41-50.....	55	17	31	0	0
51-60.....	65	11	17	3	5
61-70.....	77	4	5	20	26
71-80.....	63	0	0	23	37

The oldest case (which showed no epithelial atrophy, no fibrosis of the stroma, and no conspicuous formation of corpora amylacea) was a male, 68 years of age. Microscopic observations on the testes and pituitary gland are not available on the Vienna series and there is nothing in the autopsy protocols that might serve as an explanation of the failure of involution.

The morphological picture of these prostates need not be described since it is identical with that described earlier as the normal adult postpuberal prostate.

## SECONDARY HYPERPLASIA

The incidence of this histological change given in Table VIII shows definitely that it is associated with senility and occurs in inverse ratio to the picture described as delayed senility.

The histological picture is entirely characteristic. The acini are separated by relatively broad bands of intralobular stroma in contrast with the fine delicate bands in the normal prostate. The shape is distinctly elongated, such as has been described in the senile prostate as slit-acini. There is a relative decrease of smooth muscle fibers and increase of connective tissue of the stroma. These changes in the stroma give the impression that each acinus is separate and distinct (Fig. 19) in comparison to the richly branching, folded, intercommunicating acini of the adult prostate.

In spite of these very apparent characteristics of acinar and stromal atrophy, the epithelial cells are tall columnar. The cytoplasm is reticulated and the internal cell membrane is irregular. Secretion is found in the lumens, but few corpora amylacea. The nuclei are relatively large and vesiculated. The epithelium is thrown into numerous papillary folds but in contrast with the normal, the papillae are largely epithelial cells with only a very delicate connective tissue centrum. In localized areas the cells are of the Type 4 described above, that is, tall, narrow columnar with a dense acidophilic cytoplasm and elongated chromatic nuclei.

This secondary hyperplasia is always irregular. Some acini show taller epithelium than others, while in a few cases it is definitely focal, either a lobule or a part of the lobule only showing the change (Fig. 20). The hyperplasia in this focus may be toward the secretory type of cell or the tall, slightly anaplastic Type 4 cell.

There is in none of these types of secondary hyperplasia any reaction of the stroma, any multiplication or branching of acini and no suggestion of the formation of nodules. It is observed most commonly in the posterior lobe. The lesion is distinctly one of cellular hyperplasia of epithelium in otherwise atrophic acini.

These morphological observations indicate that during presenility and senility the prostatic tissues may be stimulated and that their response is not even, but some areas react to a greater degree or involute to a lesser degree after stimulation.

### INVOLUTION OF CASTRATION

In 1 case of the Vienna series (No. 602) a 39 year old male had submitted to a bilateral orchidectomy 5 years before death because of homosexuality. He and the surgeon realized that the operation would probably be of no value but he was anxious to secure any relief from this inversion. He died of chronic pulmonary tuberculosis.

Grossly the prostate measured 2.6 by 3 by 1.2 cm., as compared to 2.9 by 3.7 by 1.9 cm. as the average for this decade (see Table I). It was firm in consistence and on section only a few gray acini could be seen. In a few areas there was dense, fibrillar white connective tissue. The seminal vesicles were exceedingly small.

Microscopically there was an increase of connective tissue in the stroma and a decrease of smooth muscle. There was no pigmentation of the smooth muscle fibers. The acini were small, widely separated and slit-like with the long axis in the peripherohilar direction. The epithelial cells were low cuboidal or at times flattened with relatively small chromatic nuclei which occupied the greater part of the cell (Fig. 23). The marked decrease in cellular size was well illustrated by the nuclear overlapping in ordinary sections.

### PHYSIOLOGICAL CORRELATION

From the morphological observations described in the preceding sections it would appear that the physiological stimulus necessary for the maintenance of the normal prostate appears at puberty and after a period of 30 years gradually decreases for another 25 years and then is entirely absent. The histological picture of secondary hyperplasia further indicates that this stimulus or the reactivity of the prostate tissues is irregular during the late presenile period.

In all mammals that have been studied, the removal of the testis brings about an atrophy of the prostate. The general character of the changes is similar to those that have been described in senile involution in man. The appearance of the prostate in a senile male (Fig. 21), a senile rat (Fig. 22), a castrated male (Fig. 23), and a castrated rat (Fig. 24), are shown. In all of these there is a decrease in the height of the epithelium, loss of papillae, and prominence of the periacinar collar of stroma. This correlation is further supported when the prostate is examined for long periods after castration.

After 120 to 150 days a process similar to sclerotic atrophy appears, as shown in Figure 25. There is the same collar of hyalinized, relatively acellular connective tissue and irregularity in the outline of the acinus. If castrated animals are given sufficient quantities of extracts of testis<sup>31</sup> or of male urine<sup>32</sup> the normal appearance may be restored, proof that the atrophy is due to the withdrawal of one or more hormones contained in these extracts.

As shown in Figures 23 and 24, the changes of senility in man and rats\* are similar to those after castration except that the non-uniformity of the structure described during the presenile period is not apparent. In senile mice non-uniformity of structure is found from the 480th to the 720th day, and in rats from the 650th to the 725th day. Figure 26 from the prostate of a 670 day old rat shows adjacent acini, some of which show advanced epithelial atrophy and others tall columnar cells. These morphological observations indicate that the changes of senile involution are due to the same causes as those of castration — namely decrease or absence of the hormones of the testis. This assumption is supported by the finding of a decrease of hormone secretion in old age (as measured by the urinary secretion) by Funk, Harrow and Lejwa<sup>32</sup> and the report of Womack and Koch<sup>27</sup> that the testis of the calf contains more hormones than that of adult or aged animals.

By the parenteral injection of the chloroform extractives of male urine † into castrated and senile animals, pictures similar to those of secondary hyperplasia may be produced.‡ In Figure 27 the combination of tall epithelium in a flattened acinus without papillae in an injected castrated rat should be compared with the picture of secondary hyperplasia in man in Figure 19. In some cases the variation in structure is striking with marked hyperplasia in one small focus, as shown in Figure 28, entirely similar to the lesion in man (Fig. 20).

From the combined morphological and physiological evidence the

\* The prostates of 37 rats from 500 to 811 days of age have been studied. In addition, through the kindness of Dr. Jacob Furth, the prostates of 55 mice from 240 to 1140 days of age from the leukemia colony at Cornell University Medical College have been studied.

† The extraction of urine with chloroform has been carried out according to Harrow.

‡ A total of 44 white rats was injected with hormones at 10 day intervals, 5 with 50 units of "antuitrin S" after castration, 6 with the equivalent of 1000 cc. of male urine after castration, 12 senile with equal doses of "antuitrin S," and 21 senile with the same amount of male urine.

conclusion is justified that the senile involution of the mammalian prostate is due to a decrease and cessation of excretion of a hormone or hormones by the testis. Further, during the period of decrease, slight variations in the quantitative secretion combined with unequal reactivity of the tissue may result in a non-uniformity of structure.

### SUMMARY AND CONCLUSIONS

1. At puberty there is a rapid maturation of the prostate, probably due to an internal secretion of the testis which is activated by the pituitary gland.
2. The mature postpuberal prostate is maintained as a uniform structure for about 25 years, except for occasional instances of pathological involution dependent on systemic disease.
3. During the 5th decade of life involution is initiated and continues as a progressive process into the 8th decade. All the evidence indicates that this involution is the result of a decrease and cessation of the same internal secretion which appeared at puberty.
4. There are occasional cases in which senile involution is delayed for 10 to 20 years beyond the average time. There is no adequate morphological explanation for these cases.
5. During the presenile period a non-uniformity of structure is characteristic and is probably the result of irregular stimulation combined with unequal reactivity of the tissues.

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## DESCRIPTION OF PLATES

### PLATE II4

- FIG. 1. Early maturation of the epithelial cells from a child 10 years of age. The cytoplasm is more conspicuous, denser and more acidophilic than at an earlier age.  $\times 225$ .
- FIG. 2. The prostate from an individual 27 years of age showing a tall columnar epithelium with numerous papillae.  $\times 85$ .
- FIG. 3. Semidiagrammatic drawing of the prostate to show the division into lobes on the basis of the position of the urethra, deferential canal and prostatic ducts.
- FIG. 4. A section of the prostate immediately cephalad to the opening of the ejaculatory ducts. Note the white lines, radiating laterally and posteriorly, which represent the ducts. There is cystic dilatation of the acini in the most peripheral parts.
- FIG. 5. Low power photograph of an entire midsagittal section cephalad to the verumontanum. The acini are numerous and show many papillae.  $\times 2$ .
- FIG. 6. Type 1 cells (at top). The cytoplasm is slightly reticulated and each cell bulges into the lumen. The nucleus is round and moderately chromatic. There are occasional basal cells with elongated nuclei.  $\times 710$ .
- FIG. 7. Type 2 cells. The cells are tall with slight indistinctness of the luminal cell membrane. The nuclei are round and basal. The basal layer of epithelial cells is distinct with round nuclei.  $\times 710$ .
- FIG. 8. Type 3 cells. The cells are tall with a basal flattened highly chromatic nucleus. The basal cells are not evident. There is a beginning loss of the luminal cell membrane.  $\times 710$ .
- FIG. 9. Type 3 cells. The general features are the same as in Fig. 8 except that the luminal cell membrane is entirely lacking with discharge of a part of the cytoplasm into the lumen as secretion.  $\times 710$ .
- FIG. 10. Type 4 cells. The cells are moderately tall with elongated elliptical nuclei, the long axis of which is at right angles to the wall of the acinus. The cytoplasm is denser than in the other types and the luminal cell wall is smooth and distinct.  $\times 710$ .
- FIG. 11. Diagram of the cycle of secretion in the prostatic epithelium. Each cell in this diagram may be found in Figs. 6, 7, 8, and 9.

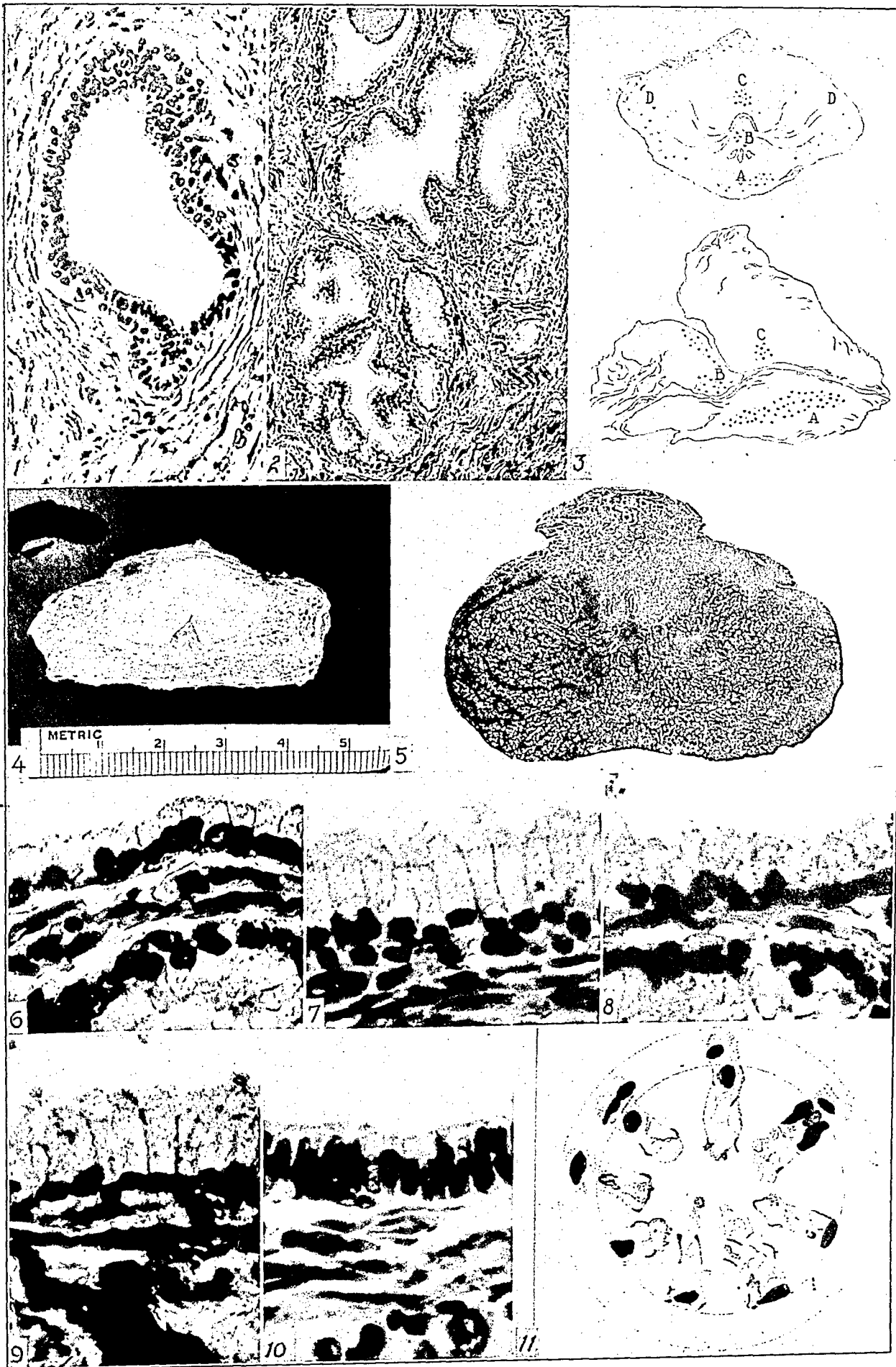


PLATE 115

- FIG. 12. Type 5 cells. The epithelial cells are cuboidal with definite cell walls and only very slight bulging of the cytoplasm into the lumen.  $\times 820$ .
- FIG. 13. Type 6 cells. The epithelial cells are low cuboidal and in thin sections there is overlapping of the nuclei. A basal cell is present only in rare areas.  $\times 820$ .
- FIG. 14. At the top there is complete atrophy of one lobule while below in other lobules there are acini with tall epithelium and numerous papillae.
- FIG. 15. Deformed acini in a senile prostate. The epithelium immediately over the connective tissue projections is flatter than in the depths of the clefts. The stroma adjacent is free of muscle fibers.  $\times 215$ .
- FIG. 16. A moderately advanced state of sclerotic atrophy. The epithelium is detached, probably due to fixation. There is a hyalinized collar about the former acinus.  $\times 169$ .
- FIG. 17. Complete sclerotic atrophy. The former acini are represented as irregular masses of loose tissue surrounded by a collar of hyalinized collagen.  $\times 83$ .
- FIG. 18. Involution of the prostate in a young man, 27 years old, with advanced pulmonary tuberculosis. The acini are flattened or irregular and the epithelium is low cuboidal.  $\times 125$ .
- FIG. 19. Secondary hyperplasia. The acini are separated and the epithelial cells are columnar. The papillae are atypical. There is moderate fibrosis of the stroma.  $\times 125$ .

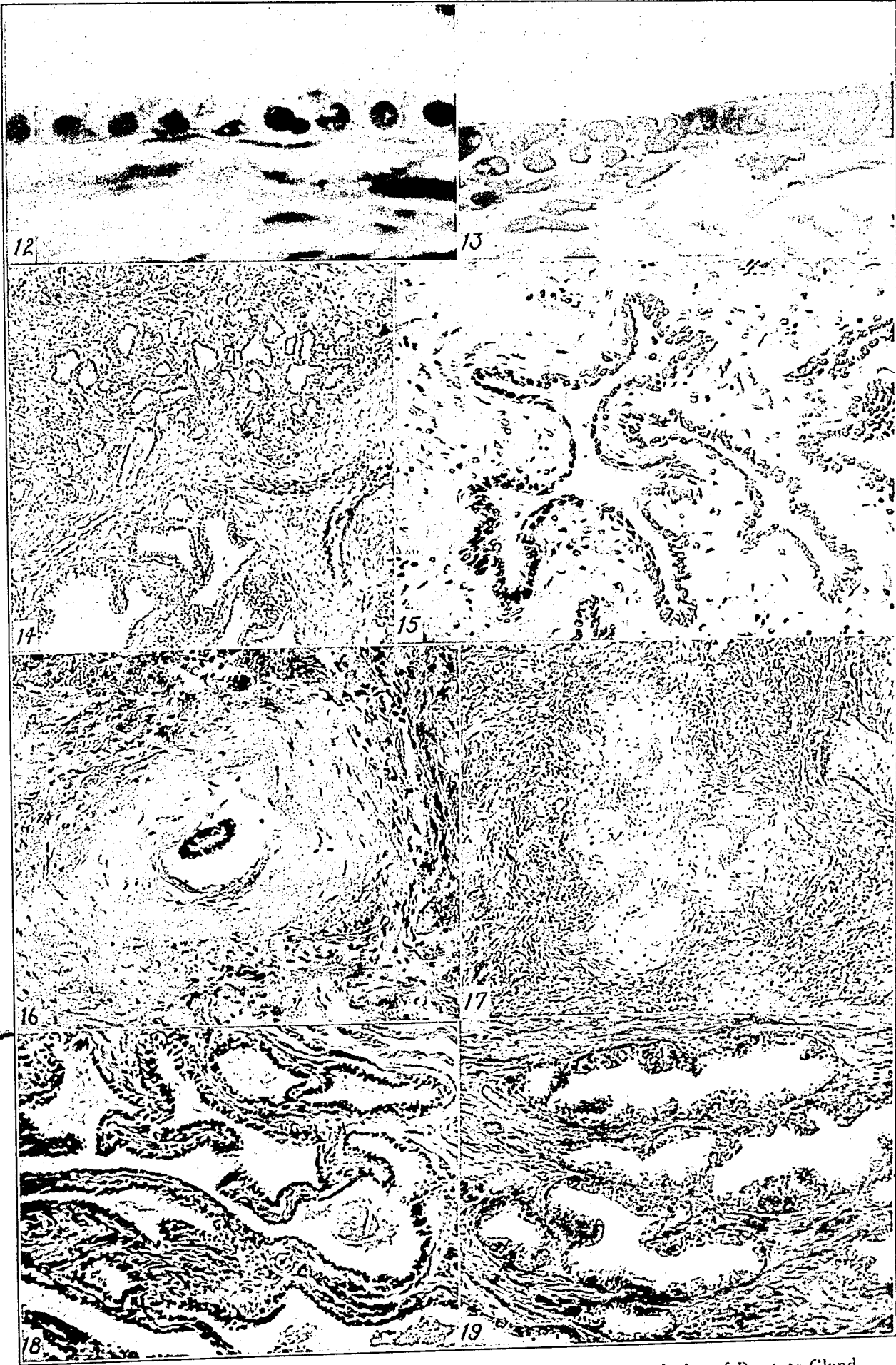
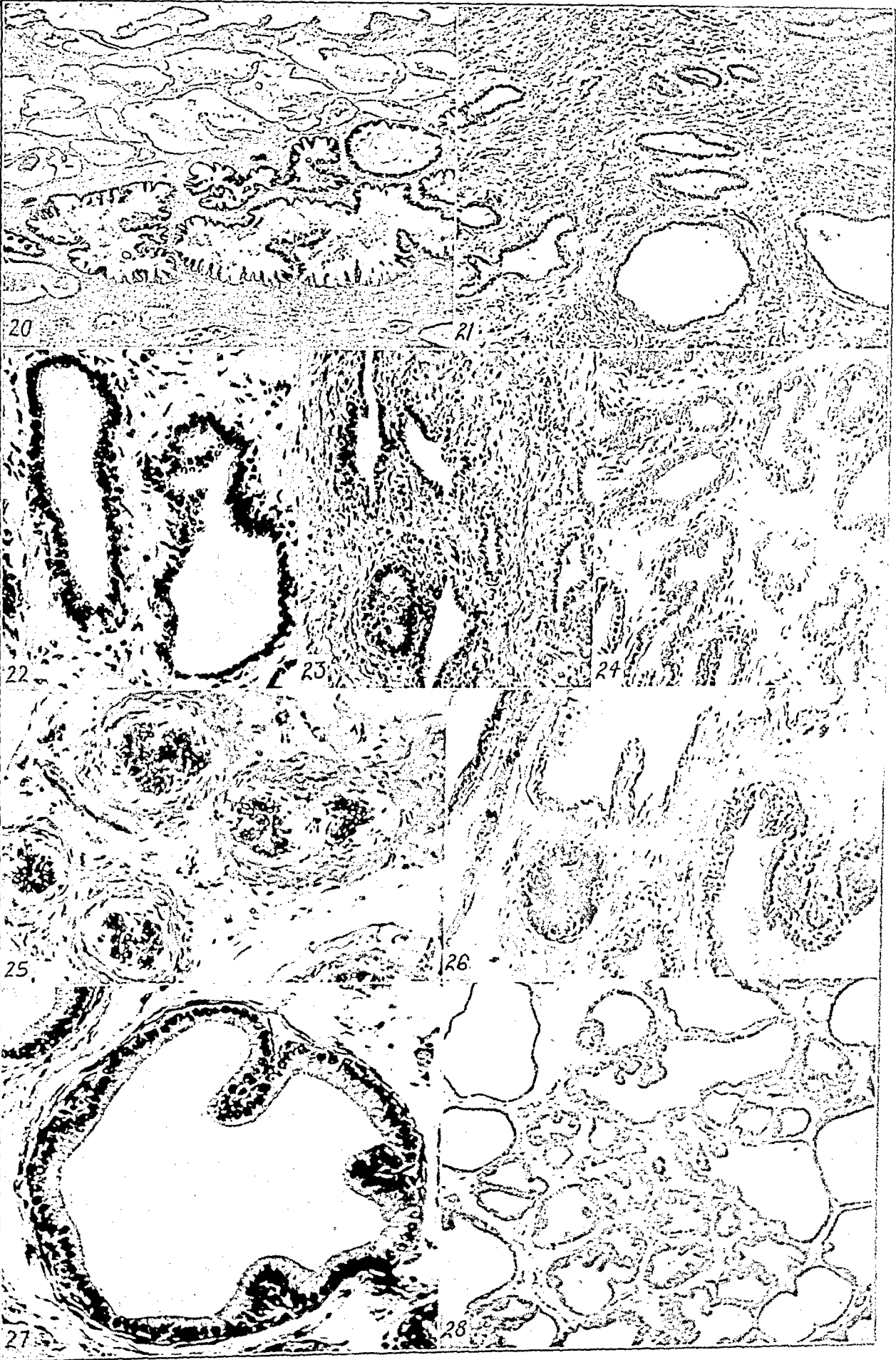


PLATE 116

- FIG. 20. Focal hyperplasia. In a local area the epithelium is tall with elliptical nuclei. There is no stromal reaction or nodule formation.  $\times 30$ .
- FIG. 21. A typical appearance of a posterior lobe of a prostate of an individual 70 years of age.  $\times 85$ .
- FIG. 22. The prostate in a rat 810 days of age.  $\times 135$ .
- FIG. 23. The prostate of a man 38 years of age who had been castrated 5 years previously.  $\times 135$ .
- FIG. 24. The prostate of a rat which had been castrated 60 days before death.  $\times 135$ .
- FIG. 25. Hyalinized collars about acini in a rat 825 days of age. Compare with Figs. 16 and 17.  $\times 135$ .
- FIG. 26. The prostate from a rat 670 days of age. In the acinus above, the epithelium is extremely flattened while in three below it is tall columnar with evidence of secretion. Compare with Fig. 20.  $\times 125$ .
- FIG. 27. A castrated rat which had received for 10 days before death three bird units of the male sex hormone each day. The epithelium is tall but slightly irregular and the typical peripheral lighter areas are not present.  $\times 200$ .
- FIG. 28. A senile rat 800 days of age which had received ten injections of ten bird units of male sex hormone at intervals of 10 days. Note the irregularity in the structure of the acini and the numerous papillae in the central group.  $\times 55$ .







## CERTAIN CYTOPLASMIC INCLUSIONS OF LIVER CELLS \*

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On routine examination of liver sections our interest has been aroused by the rather frequent occurrence of certain, well characterized cytoplasmic inclusions. Although easily seen, even with ordinary stains, they seem to have attracted little attention and to have escaped the recognition of experienced pathologists. They are not described in standard textbooks of pathology, histology or cytology. Mallory,<sup>1</sup> however, in his classical paper on necroses of the liver (1901) has accurately described and pictured these bodies (Plate XVI, Fig. 1, and Plate XVII, Fig. 1), and since we have found but one other reference to them his description may be cited in full.

"In a majority of the early cases the protoplasm of the liver cells contains numerous small and large vacuoles in which are single small hyaline globules; single, sometimes multiple, coarse, and fine threads; and occasional networks, all of which stain deeply with eosin and with Weigert's fibrin stain. With other stains they react exactly as fibrin does. The hyaline globules are not threads cut across; whether they really are fibrin is difficult to prove. They often seem to precede the formation of the threads and networks of fibrin."

It would seem that Mallory regarded these bodies as an accompaniment of early degeneration, ending in necrosis of the liver cells.

We have been able to locate but one other short reference to these structures. Taniguchi<sup>2</sup> (1931) describes in liver cells three types of cytoplasmic inclusions as follows:

*Type 1:* Spherical bodies, varying in size from a round mitochondrial granule to that of a nucleus, homogeneous and with sharp contours. They stain deeply with Heidenhain's iron hematoxylin after fixation in Regaud's fluid and can be distinguished, though less clearly, in hematoxylin-eosin preparations. They do not stain with Sudan III.

*Type 2:* More or less elongated oval bodies which are regarded as a modification of Type 1. They are infrequently found.

\* Received for publication March 25, 1936.

*Type 3:* Comparatively narrow, long and short, thread-like, slightly bent, sometimes spindle shaped bodies. They are derived from swollen, thread-like mitochondria.

The material for Taniguchi's study was obtained from surgical biopsies. The bodies described as Type 1 were found in 27 of 94 cases. The photographic illustrations accompanying the paper show, though not very clearly, spherules and filaments.

We have been interested in making a somewhat more extended study of these bodies.

### DESCRIPTION OF THE INCLUSIONS

(A) *Spherical Bodies:* Our observations in the main confirm the description given by Mallory. The spherules vary in size from that of a small micrococcus up to 4 or 5  $\mu$  in diameter, the majority ranging from 1 to 3  $\mu$ . They lie in a large vacuole which, with the usual staining methods, is optically empty or contains only a little shredded material. When the vacuole is contiguous to a nucleus the nuclear membrane may be indented by it. There may be but a single spherule or the entire cytoplasm may be riddled with vacuoles, each containing a single body, so that the cell has a cribriform or porous structure. Adjacent vacuoles may coalesce into large spaces containing several spherical inclusions. When they are on the surface they may rupture, discharging the inclusion body into the space between liver cell and sinus wall.

### *Staining Reactions*

With *hematoxylin-eosin* staining, the spherical bodies are acidophilic, but the color is unlike that of the cytoplasm or of the erythrocytes, having a brownish yellow cast. They are sharply contoured, moderately refractive, and entirely homogeneous without any suggestion of internal structure.

With the *Gram* stain the methyl violet or gentian violet is retained, if decolorization is not pushed too far. The degree of Gram positiveness is about the same as that of the nucleolus.

*Mallory's phosphotungstic acid hematoxylin* stains the spherules very selectively a bluish black.

After formalin fixation, staining with *phosphotungstic* and *hematoxylin* demonstrates that the spherule is surrounded by a homo-

geneous capsular material which stains a dull brick red and completely fills the vacuole (Fig. 3).

*Heidenhain's iron hematoxylin* also brings the bodies out sharply but they are resistant to decolorization with ferric chloride.

With *azocarmine*, *anilin blue* and *orange G*, the bodies take a dull orange color similar to that of the erythrocytes.

With *methylen blue-pyronin*, both spherules and rods take only a faint yellowish stain. With *mucicarmine* the globules do not take the mucin stain.

*Masson's trichrome* stain shows the spherules to be red, in contrast to the dark violet gray of the cytoplasm. The nucleoli are bluish black. Contents of the vesicle are pale blue. There is no unstained vacuole. The bluish material is sometimes homogeneous, sometimes finely granular (Fig. 4). The rods stain orange-red and lie in a clear vacuole without the bluish staining matrix.

*Mallory's anilin blue* collagen stain shows about the same picture as that of Masson's, except that the spherules are orange-red and the bluish matrix is less sharply brought out.

With *Mann's* stain the spherules are dull red to bluish purple, depending on the degree of differentiation. Filaments stain about the same. Contents of vacuoles remain unstained (Fig. 1).

With *Giemsa*, they are stained a rose pink.

The most selective stain that we have found is *Laidlaw's* method for demonstrating virus inclusions.\* By careful differentiation with orange G alcohol the cytoplasm of the liver cells may be decolorized to a grayish blue: the spherical inclusions retain the fuchsin and stain an intense crimson. The red blood cells are a tawny orange-red (Fig. 2). While Laidlaw's method calls for fixation in acetic

#### \* LAIDLAW'S METHOD FOR STAINING INCLUSION BODIES

1. Fixation in { saturated aqueous corrosive sublimate ..... 100 cc.  
glacial acetic acid ..... 5 cc.
2. Embed in paraffin and cut at 3  $\mu$ .
3. Deparaffinize in xylol, absolute and 95 % alcohol and rinse in water.
4. Stain in Weigert's iron hematoxylin (2 %) 5 minutes.
5. Differentiate in 0.5 % acid alcohol.
6. Rinse in tap water followed by distilled.
7. Stain in 1 % aqueous acid fuchsin 5-15 minutes.
8. Rinse in distilled water.
9. Mordant in 1 % phosphomolybdic acid for 30 seconds.
10. Rinse in distilled water.
11. Differentiate in 0.25 % orange G in 70 % alcohol.
12. Dehydrate, clear and mount in balsam.

acid bichloride, Zenker's fixation without acetic acid gives equally brilliant pictures.

The bodies are not stained by *scharlach R*.

(B) *Rod Shaped and Filamentous Structures*: These occur almost always in association with the spherical bodies, but far less frequently (only in about one-fifth of the cases). They also lie in clear vacuoles and assume a variety of forms — simple bacillus-like rods, beaded or fuzzy straight rods, or long curving fibrils, sometimes looped upon themselves. Small crossing or interlacing filaments may be seen within the same vacuole.

Not infrequently the fibrils seem to be formed directly on or from the spherical body; the spherules may lie in the middle or at the end of the rod. But in many of the vacuoles containing rods or filaments, no spherical body can be discerned.

The staining reaction of these filaments and rods, as Mallory pointed out, is the same as that of fibrin. They retain the gentian violet in the Gram-Weigert stain, and stain bluish black with phosphotungstic acid hematoxylin. They are also brought out by the Heidenhain iron hematoxylin method and by the Laidlaw stain.

### General Incidence

In all, 562 sections of liver from human cases have been studied. The spherical inclusions were found in 175 cases, or 31.1 per cent. Many blocks were recut and stained with phosphotungstic acid hematoxylin, and in these preparations the bodies are more easily recognized. Their incidence in the hematoxylin-eosin sections was therefore somewhat lower (23.7 per cent) than in the 124 sections stained with phosphotungstic acid hematoxylin, which gave 43.6 per cent of positives.

Included in the 562 cases are 391 adults, 124 children, and 47 newborn infants and stillbirths. The percentage incidence in the different age groups is shown in Table I.

TABLE I

Age	2 wks.	2 wks.— 1 yr.	1-5 yrs.	6-10 yrs.	11-20 yrs.	21-30 yrs.	31-40 yrs.	41-50 yrs.	51-60 yrs.	61-70 yrs.	71-80 yrs.	80+ yrs.
No.	47	46	30	18	30	42	66	79	104	65	32	3
No.†	14	6	7	6	9	14	27	23	40	17	10	2
%†	29.7	13.0	23.3	33.3	30.0	33.3	41.0	29.1	38.4	26.1	31.2	66.6

There is, as the analysis shows, no significant difference in the various age groups. The spherules were found once in a stillborn full-term infant, and in several prematures.

### *Occurrence in the Human Fetus*

Through the courtesy of Dr. Coler of the Sloane Hospital, we have been able to obtain material from human fetuses from 5 months to term. The sections stained with phosphotungstic and hematoxylin showed an astonishingly high incidence of inclusions.

<i>Age of Fetus</i>	<i>No. Examined</i>	<i>Positive</i>	<i>Negative or Doubtful</i>
5 mos.	7	7	..
6 mos.	7	4	3
7 mos.	7	6	1
8 mos.	6	5	1
Full term	<u>7</u>	<u>4</u>	<u>3</u>
	34	26	8

The failure to identify the bodies in a few of the cases may have been due to poor preservation of the tissue. In 8 of the 34 cases, that is in about the same proportion as in the postnatal livers, the spherules were associated with rods and filaments.

### *Relation to Possible Postmortem Changes*

The bodies seem to be relatively little affected by autolytic changes, and they do not become more numerous after death. They have been found as early as 45 minutes postmortem and as late as 53 hours. The incidence of positive findings at various periods after death is shown in Table II.

TABLE II

Hours postmortem	2	2-6	6-12	12-24	More than 24
Total autopsies .....	26	165	130	146	43
No. showing globules .....	6	50	45	39	16
No. showing rods .....	1	12	13	9	4
% globules + .....	23.1	30.3	34.6	26.7	37.2
% rods + .....	3.8	7.2	10	6.2	9.3

Although there are slight variations in the different groups, there is no trend toward a definite increase or decrease in the incidence, as the period elapsing after death becomes greater.

*Relation to Other Diseases*

We have attempted to correlate the occurrence of the spherules with various general diseases such as arteriosclerosis, nephritis, and acute and chronic infections. As might have been anticipated from the rather uniform age distribution and the presence of the bodies in the fetal liver, no such correlation seems to exist. The bodies have been present in all sorts of pathological conditions, and a detailed analysis of our material from this point of view would be futile. The only reservation to the above statement is in the case of malignant disease. Among the 562 cases examined, 111 had malignant tumors, and of these 24 showed liver inclusions — an incidence of 21.6 per cent as against an incidence of 33 per cent in the 451 non-tumor controls. The significance of this lowered occurrence is not apparent, but the difference would appear to be too great to ascribe to chance.

Not only is there no clear-cut correlation with general diseases, but we have been able to find no constant association with any particular type of liver lesion. The inclusions are found frequently in otherwise normal appearing cells. When they are very numerous the cytoplasm of the cells containing them has a spongy or cribriform appearance. They are always more abundant in the central portion of the lobule and often seem to be especially numerous when the liver cells are atrophic, or in the presence of central congestion. But they are quite definitely not associated with necrosis of the cells and we cannot interpret their presence as an indication of early degeneration or cell death. On the other hand, they may often be recognized in cells that have recently undergone necrotic changes. We have not found them in the neoplastic cells of primary liver cell carcinoma.

*Occurrence in Laboratory Animals*

In monkeys the spherical inclusions have been found in 1 of 5 livers examined. In guinea pigs they have been seen in 8 out of 44 livers — slightly less frequently than in human livers. In 7 ferret livers they were present in every instance. Rods and filaments, however, have not been observed.

On the other hand, we have searched in vain for these bodies in the livers of rabbits, dogs, cats, pigs, rats, mice, chickens or ducks.

## DISCUSSION

It has not been possible to arrive at any final conclusion as to the nature of the spherical inclusions. Several possible interpretations come to mind and may be discussed briefly:

1. They are some unknown structural component of the cell, like the Golgi net, centrosome, or mitochondria.
2. They are secretory products.
3. They are degenerative products, akin to the colloid droplets in degenerative renal epithelial cells.
4. They are cytoplasmic "virus inclusions."

It is difficult to believe that these structures represent some unknown organs of the cell. Were such the case, one would expect to find them in every cell, certainly in every liver and in every species. Nor is there any reason to believe them to be derived from known cytoplasmic constituents. They stain quite differently from mitochondria, persist long after the mitochondria have been destroyed by autolysis, and are not affected by acid. There is nothing to suggest that they are related to the Golgi-net or centrosome, and they are most certainly not due to extrusion of nuclear chromatin.

We can find no particular evidence for or against the possibility that they represent a secretory product of the liver cells. The fact that with Gram and phosphotungstic acid hematoxylin the staining is like that of fibrin, brings up the thought that they may be associated with the production of fibrinogen. But the staining for fibrin is far from being specific and it would require much more evidence to justify such an assumption.

Against the secretory nature of the product is the observation that only a single body may be present in the cell. We can recall no instance in which secretory granules were not multiple. Perhaps it may be taken as a point also against the secretory nature of these bodies that they are not found in all human livers, as one might expect if they represent a normal activity of the liver cells, and that they are not demonstrable in the livers of dogs, rats, rabbits, cats, chickens or ducks.

Against the view that the spherules represent a degenerative alteration in the cytoplasm is the fact that they are often found in cells that show no other evidence of injury. It is true that they are most numerous in the central portions of the liver lobules, where atrophy

or necrosis accompanying central congestion is so frequently seen. Doubtless also, their presence in great number, with the resulting spongy rarefaction of the cytoplasm, may lead to the disintegration of some of the cells. On the other hand, the bodies may be found in cells adjacent to the portal spaces, and in this situation the liver cells often show no evidence whatever of damage.

The last possibility which comes to mind is that the bodies are of the nature of virus inclusions. This assumption would lead of necessity to the view that a large proportion of human livers harbor a non-pathogenic virus or viruses, and that similar viruses occur in the livers of monkeys, guinea pigs and ferrets. The fact that we have found the inclusions in the liver cells of the fetus would also logically involve the assumption that the hypothetical virus is transmitted to the embryo.

The only evidence in favor of these bodies being virus inclusions is (1) their morphology, which is not unlike that of certain cytoplasmic inclusions, such as the Guarnieri bodies (*cf.* Cowdry, E. V., *J. Exper. Med.*, 1922, 36, 666, Fig. 33); and (2) the staining reactions. We have had opportunity to compare the liver cell inclusions with cytoplasmic virus inclusions in the various tissues of ferrets experimentally infected with a virus now being studied by Dochez, Slanetz and Smetana.\* The staining of the liver cell inclusions is practically identical with that of the virus inclusions, but the latter are not surrounded by a stainable halo.

It would be of interest to obtain experimental support for the virus theory, as the demonstration of such a frequently occurring, latent, non-pathogenic virus would be of great theoretical importance. Unfortunately we have thought of no obvious way of attacking the problem experimentally at the present time.

As regards the rod-like and filamentous inclusions, we agree with Mallory that they are probably fibrinous in nature. They occur in about one-fifth of the livers containing the spherical inclusions, but only in association with them. They may occupy the same vacuole as the spherical bodies, and indeed one frequently gets the impression that filaments have formed on the spherical bodies as a nucleus. But often the rods and filaments occur in vacuoles that contain no spherical body. The only plausible explanation for their occurrence is that coagulable fluid from the plasma has seeped into the

\* We are indebted to Dr. Smetana for placing this ferret material at our disposal.



vacuoles from the adjoining sinusoids and has crystallized out within the vacuoles. Why this does not occur in every case is not easy to understand.

#### SUMMARY AND CONCLUSIONS

A study has been made of certain spherical, rod-like and filamentous cytoplasmic inclusions that occur frequently in the liver cells of man, monkeys, ferrets and guinea pigs. They have not been seen in the livers of other laboratory animals. While no decision has been reached as to their nature and significance, various possible interpretations are discussed.

#### REFERENCES

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2. Taniguchi, A. Cytological studies of the liver cells. *Tr. Jap. Path. Soc.*, 1931, 21, 260.

## DESCRIPTION OF PLATES

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### PLATE 117

- FIG. 1. Liver cells containing spherical and filamentous cytoplasmic inclusions. Mann's stain.
- FIG. 2. Spherical inclusions. Laidlaw's stain.
- FIG. 3. Spherical inclusions surrounded by a homogeneous, slightly reddish substance. Formalin fixation, phosphotungstic acid hematoxylin stain.
- FIG. 4. Spherical inclusions surrounded by bluish staining material. Masson's trichrome stain.



1



2



3

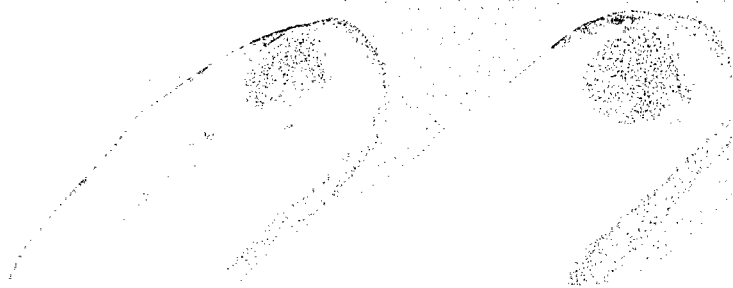


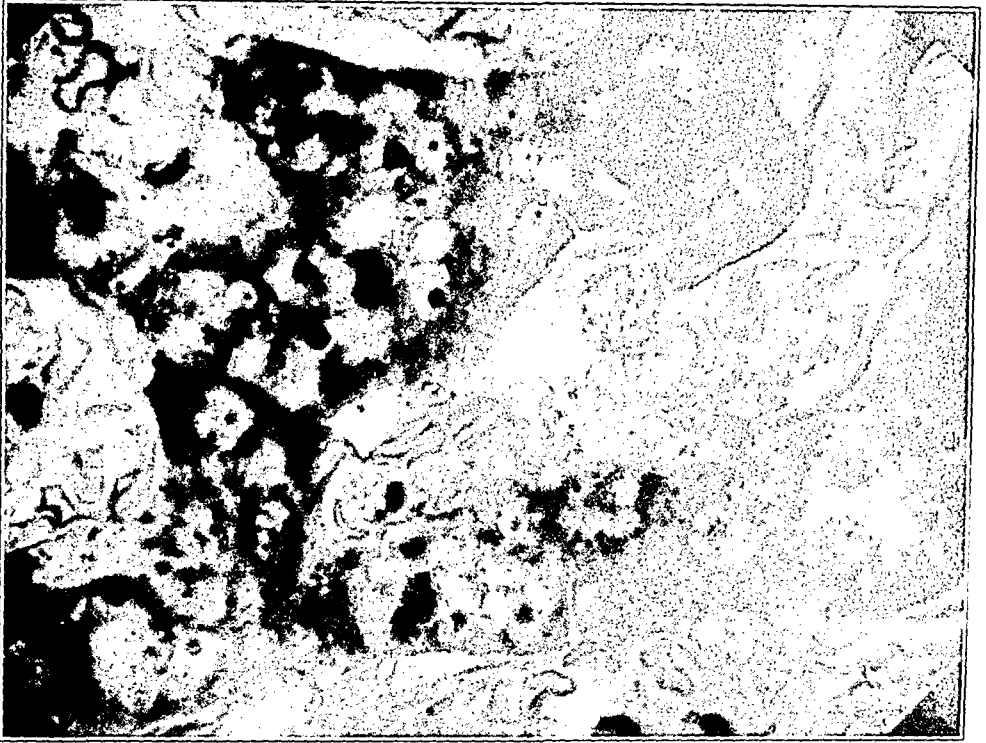
4

PLATE 118

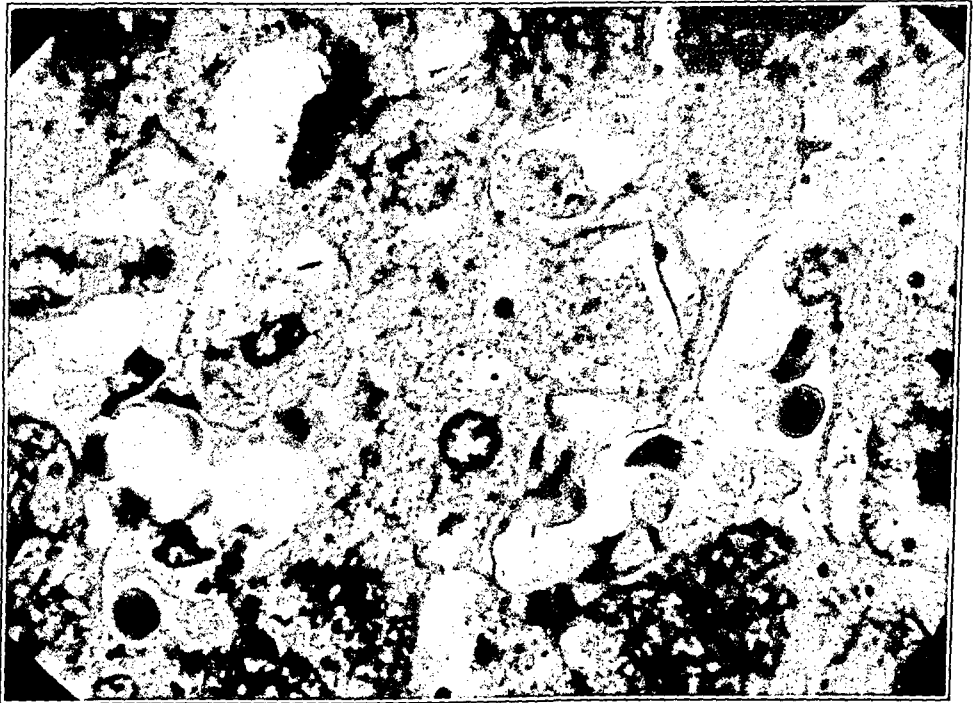
FIG. 5. Spherical inclusions in liver cells. Note absence of degenerative changes in nucleus. Phosphotungstic acid hematoxylin stain.  $\times 1050$ .

FIG. 6. Spherule with long filament attached is seen to right of center. Smaller rod-like structures and spherules are seen in other cells. Phosphotungstic acid hematoxylin stain.  $\times 1050$ .





5



6



# THE RELATION OF AGE AND HYPERTENSION TO THE STRUCTURE OF THE SMALL ARTERIES AND ARTERIOLES IN SKELETAL MUSCLE \*

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This study was made to determine whether or not age or chronic hypertension produces any characteristic changes in the structure of the small arteries and arterioles in skeletal muscle.

Many observations of the arterial changes in hypertension have been made. In 1868 Johnson examined the small arteries in 10 cases of chronic Bright's disease. Since he did not consider that the vessels of the kidneys alone could increase peripheral resistance, he also studied those of the pia mater, brain, intestine, subcutaneous tissue and muscle, and stated that in each the media of the arteries was hypertrophied. Four years later Gull and Sutton described what they called "arterio-capillary fibrosis" in Bright's disease. This was said to consist of a hyalin-fibroid substance which was formed external to the muscular layer. It was thought to be a morbid change. These authors stated that the arterioles were often very much thickened and tortuous. They drew an outline of the vessel with a camera lucida and measured the ratio of the thickness of the wall to the diameter of the lumen. In cases of kidney disease the usual finding was a lumen equal to twice the width of the wall. A normal relationship was not established. The muscle was said not to be increased. They thought that the lesions in the arterioles of the kidneys were identical with those found in the arterioles of other organs. This alteration of the arterioles described by Gull and Sutton does not correspond with anything seen when modern technical methods are used.

In 1877 Ewald confirmed Johnson's findings. He examined only the vessels of the pia mater in the region of the pons and also determined the wall to lumen ratio which in normal individuals was 0.1 to 0.3:1.0. In cases of contracted kidneys with hypertrophy of the left ventricle the ratio rose from 0.3 to 1.2:1.0. The increase in

\* Received for publication February 6, 1936.

the thickness of the wall was due to a simple increase in the number of muscle fibers.

Jores, in 1904, examined the small arteries in cases of contracted kidneys with cardiac hypertrophy. He was unable to find any change whatever in the vessels of skeletal muscle except in 1 case of adhesive pericarditis and aortic insufficiency in which the circular muscle of the media appeared to be thickened.

Evans, in 1920, described diffuse hyperplastic sclerosis of the renal arteries in chronic nephritis. Under this term he included the fatty or hyaline swelling of the intima, hypertrophy of medial muscle and perivascular fibrosis. He also examined the arterioles of various other organs including the skeletal muscle in 31 cases. In the arterioles of muscle he found changes in only one patient, an irregular intimal thickening which he interpreted as being a syphilitic lesion.

In a study of 72 cases of hypertension Fishberg found lesions in the small arteries in 100 per cent of the kidneys. Arteriolosclerosis was present in the spleen in most of the cases. It was found in a smaller percentage of the cases in the pancreas, liver, adrenals, brain and gastro-intestinal tract. Arteriolosclerosis was never observed in the skeletal muscle in this series.

In 1927 Fishberg described the necrotizing arteritis, endarteritis obliterans, and arteriolosclerosis seen in the kidneys in acute and chronic glomerulonephritis. He was unable to find changes in the muscle of the media in early nephritis except in the necrotizing endarteritis seen occasionally in acute glomerulonephritis. In chronic nephritis he found a thickening of the medial muscle layer which was often most marked in the arcuate and interlobular arteries. He thought that this was due to hypertrophy and hyperplasia of the muscle cells without fibrosis. He did not state what staining methods were used. In renal arteriolosclerosis and chronic glomerulonephritis of long standing the muscle of the media was usually atrophied.

Branch and Linder in 1926 studied the arterioles of the skeletal muscle in 6 cases of chronic nephritis and were unable to demonstrate any changes. Theodore Fahr has stated that hypertension is secondary to renal arteriolosclerosis. He found no changes in the arterial systems of the skeletal muscle or skin.

In 1928 Keith, Wagener, and Kernohan described what they called "malignant" hypertension, and noted widespread arterial lesions in these cases. In the following year Kernohan, Anderson



and Keith studied the arterioles of pectoral muscle in cases of hypertension, and in 1931 Keith, Barker and Kernohan reported studies of the arterioles from 143 patients with hypertensive disease. Skeletal muscle was chosen for study because it comprises 30 to 40 per cent of body weight, and pectoral muscle was chosen because it is easily accessible for biopsy. It was thought that a generalized vascular change would of necessity affect these vessels. Arteries having an outside diameter of from 25 to 100 microns were measured and the ratio of the thickness of the vessel wall to the diameter of the lumen was determined. The normal ratio was found to be 1.0:2.0 with a variation of 1.0:1.7 to 1.0:2.7. They considered the most striking changes in hypertension to be a thickening of the media which was interpreted as due to an increase in the number of muscle fibers. They found no increase in the connective tissue of the media and thought that the medial change was due entirely to a proliferation of muscle. The internal elastic lamina was also hypertrophied. In some cases it was split but usually not to a pronounced degree. They also found proliferation of the lining endothelium of the intima which was sometimes accompanied by proliferation of the subendothelial connective tissue. They saw no degenerative changes which they could attribute to senile retrogression. They classified their cases as "benign," "severe benign," and "malignant" hypertension. In benign hypertension the wall to lumen ratios were 1.0:1.1 to 1.0:1.8, with an average of 1.0:1.4. Cases of severe benign hypertension and malignant hypertension showed practically the same ratios, an average of 1.0:1.1 with variations from 1.0:0.9 to 1.0:1.7. These authors use the term "arteriolosclerosis" to describe these medial changes though most writers restrict the use of the term to those arterioles in which intimal changes predominate.

In 1932 Horine, Weiss and Beard reported a case of an 88 year old negro who was first seen in congestive heart failure. The blood pressure was then 130/80. Biopsy of pectoral muscle 9 days before death showed a wall to lumen ratio of 1.0:0.96. At postmortem the vessels of the same muscle had a ratio of 1.0:0.93. There was also reduction of the relative size of the lumen in the small arteries of the heart, liver, spleen, pancreas and kidney.

In a study of the arterioles of pectoral muscle of 153 of 375 patients having essential hypertension, Murphy, Grill, Pessin and Moxon found moderate to advanced hypertrophy of the media in the art-

erioles of 57 of 124 of the persons having high blood pressure and arteriosclerotic disease. Hypertrophy of the medial muscle was observed in all of the 29 patients who had malignant hypertension. The degree of hypertrophy varied from "slight" to almost complete closure.

Scott, Seecof and Hill graded arteries of skeletal muscle in patients suffering from arterial hypertension according to thickening of the vessel wall, hyalinization of the vessel wall, other changes such as fatty, fibrous and necrotizing lesions, and size of lumen. They were able to demonstrate arteriolar lesions in skeletal muscle in a very high percentage of individuals dying under 46 years of age of diffuse vascular disease with hypertension. In 68 per cent of 386 autopsied cases there was a relation between the severity of the arteriolar lesions in the kidney and those of skeletal muscle. Necrotizing arteriolar lesions were not noted in skeletal muscle. They found that the lesions in biopsy specimens agreed closely with those found in a postmortem specimen of the same muscle.

Pilcher and Schwab, following the work of Keith and his co-workers, made measurements of arterioles of the kidneys, liver, pancreas, spleen and myocardium in 15 cases of hypertension. There was uniformly a reduction in the wall-lumen ratio except in the myocardium where the ratio was lowered in 4 cases. These authors, however, believe that the increased peripheral resistance cannot be regarded as resulting from thickening of the walls of the arterioles. They cite the absence of true generalized arteriosclerosis, the fact that clinically hypertension precedes the arteriolar changes, the acuteness of the condition in acute nephritis and urinary obstruction, and the variability of arterial pressure as proof that hypertension must result from a functional vasoconstriction of the arterioles, and that the arteriolar lesions are the result of the elevation of blood pressure.

The part that arterioles of the somatic area may play in the regulation of blood pressure is by no means established. Dawson, von Bonsdorff and others have shown that the blood pressure is fairly well sustained until the small muscular arteries are reached. Landis has determined the capillary pressures directly, and Voldeng, by making indirect measurements of the capillary pressures, showed that the values in normal subjects and in persons suffering from hypertension are very nearly the same. It may be seen therefore

that the pressure gradient is greatest in the small arteries and arterioles. When Jansen, Tams and Achelis put tourniquets on three extremities and measured the blood pressure on the free arm the blood pressure rose only 6 per cent or not at all, while the same procedure in hypertensives caused a sustained rise in blood pressure of 15-20 per cent. This phenomenon was interpreted by them to indicate that the splanchnic vessels in patients with hypertension lose their ability to distend and thus compensate for constriction of peripheral vessels. It is more probable that the greater rise was due to the more exaggerated responses to painful stimuli made by persons with hypertension. Steele and Kirk point out the fact that observations on patients with Buerger's disease confirm the experimental findings that no relation exists between the obstruction of the arteries of extremities and the level of systemic blood pressure.

The rise in arterial pressure following the injection of adrenalin is caused by constriction of the arterioles of the skin and abdominal viscera. At the same time there is dilatation of the arterioles of skeletal muscle and probably also of the heart muscle. Because of the relatively numerous vasomotor fibers to the arterioles of the skin and splanchnic area, it is probable that the contraction of the arterioles of these regions may account for some other elevations in blood pressure.

Jansen and his co-workers found also that cold colonic irrigations were followed by a rise in blood pressure of 22.5 per cent while a cold bath caused a rise in blood pressure of only 8 per cent. Ligature of the renal arteries of animals causes no rise in blood pressure, but ligation of the superior mesenteric artery causes a marked rise in blood pressure. The action of the arterioles in the splanchnic area has a profound effect on the level of arterial pressure.

In 1924 Brogsitter investigated the mesenteric arteries in normal individuals and in 33 cases of persistent hypertension. In some of the larger arteries he found nodular, cellular and fibrous intimal proliferations and homogeneous areas in the media. In others there was a localized thickening of the adventitia with reduction of muscle in the media and a cellular intimal proliferation. He interpreted these lesions as the healing of an inflammatory process with scar formation. Severely altered vessels were found in only a few persons. Besides the vessels with changes there were always others of the same size in the same mesentery which were entirely normal.

There was no constant relation between the arterial changes and the presence of hypertension.

Moldenhauer determined the blood pressure in patients having malaria and found that during the cutaneous vasoconstriction of each chill the arterial pressure rose only 5 to 20 mm. Hg. Steele and Kirk found that the attacks of paleness and numbness of the skin in hypertensive individuals resulting from constriction of arterioles are not accompanied by rise in blood pressure. They measured skin temperatures on nine hypertensives and found that the form of the curve of surface temperatures in a 24 hour period did not differ from the curve obtained from normal individuals and that the level of the two groups was approximately the same. When the arterial pressure is high, some increase in tone must develop in order to maintain a normal flow of blood through the skin. The latter is known to be true by the fact that the skin temperature is normal. If there were no increase in tone, excessive loss of heat would result in the presence of high arterial pressure. These workers regard the increase in tone of skin vessels as dependent on, rather than the cause of, general arterial hypertension. Watanabe was able to demonstrate sclerosis of the small arteries of the skin in only 21 of 116 persons when 4 specimens of skin from each subject were studied. In only 2 of the cases were there findings in more than 1 specimen and in only two sites in those. Of the 21 persons, only 2 had myocardial hypertrophy and these had emphysema. From these findings it may be seen that constriction of the cutaneous vessels alone has little or no effect on the arterial pressure. Their structure is not altered in hypertensive states.

The term "arteriole" does not have an exact meaning in the literature. Evans defined an arteriole as an artery with a media of two or three muscle cells in thickness. The afferent glomerular arterioles have an outside diameter of 25 to 45 microns. Maximow and Bloom described an arteriole as an artery having a simple endothelial lining lying next to the internal elastic lamina, and a media that is composed only of muscle. The adventitia equals the media in thickness. They state that the internal elastic lamina becomes thinner as the vessel becomes smaller and becomes invisible when the vessel has a diameter of 62 microns. When the vessel becomes 27 microns in thickness it loses its muscular coat. The adventitia loses its elastic fibers and becomes transformed into a thin collage-

nous membrane with scattered fibroblasts and gradually passes over into the perivascular cells of the capillaries.

### PERSONAL OBSERVATIONS

For this study 137 specimens of pectoral muscle taken from routine autopsy material were examined. Arteries having an outside diameter of 15 to about 150 microns were studied. Some of the vessels with a diameter above 100 microns would be called small arteries by many authors. The same criteria that Bell and Clawson used in their study of hypertension were employed to determine the presence of hypertension. Persons with heart weights of over 450 gm. in females and over 500 gm. in males in the absence of valvular disease, glomerulonephritis, pericarditis or hyperthyroidism were taken as cases of hypertension. All persons in whom the systolic blood pressure was known to have been 150 mm. Hg. or more were included in the hypertensive group regardless of the weight of the heart. There were 32 cases in the hypertensive group. The heart weight was above the limits of normal weight in 20 cases. There were no instances of normal blood pressures when the heart weight was above normal. In 14 cases the blood pressures were not recorded, and in 12 cases the blood pressures were above 150 mm. systolic, although the heart weights were less than 450 gm. in the female and 500 gm. in the male. There was a total of 40 females and 97 males in the entire series. Muscle was examined from one stillborn infant. Pieces of pectoral muscle were fixed in 10 per cent formalin, embedded in paraffin, and stained with Heidenhain's modification of Mallory's aniline blue collagen stain. This stain distinguishes muscle cells sharply from all types of connective tissue. Muscle cells are colored red; collagenous, elastic and reticular fibers are blue. Some of the sections were also stained with hematoxylin-eosin, Weigert's elastic tissue stain and the Van Gieson stain.

There is no diameter at which the structure of the vessel wall suddenly changes and permits one to distinguish an arteriole from a small artery. Arteries with an outside diameter of less than 150 microns have a simple endothelial lining which lies directly upon the internal elastic lamina. No nuclei or fibers were seen between the endothelium and the internal elastic lamina in any instance. The internal elastic membrane was not seen to be split in any of

the vessels of skeletal muscle up to 100 microns in diameter. Hyalinization of the intima was never observed.

The Mallory-Heidenhain (azocarmine) stain is used to great advantage in the study of the media, for it stains not only the coarse collagenous fibers, but also the reticulum. (Mallory's aniline blue stain does not stain reticulum fibers.) It may be seen here, as Mallory and Parker showed by the use of silver impregnation methods in leiomyoblastomas, that each muscle cell is separated from the surrounding ones by a layer of reticulum. This may be seen in Figure 1. The most marked variations encountered in the small arteries and arterioles of skeletal muscle were in the relative amounts of connective tissue fibers in the media. Mallory and Parker state that this reticulum is collagen which is divided into fine strands and thin layers. When in this finely divided state reticulum is stained by silver impregnation methods; when it occurs in coarse bundles it stains like collagen. They found that with necrosis of the muscle cells of smooth muscle tumors the reticular fibers become compacted and give the staining reactions of collagen.

In my material no areas of hyalin were observed in the media of any of the small arteries or arterioles. All of the fibers run in a circular or longitudinal direction. Radial T or Y shaped fibers like those Dietrich described in the larger arteries are not found in the arterioles. There are few elastic fibers in the media. Fibroblasts are not seen in connection with the reticulum. In cases of marked fibrosis of the media the muscle cells disappear and the wall is almost completely composed of blue staining material when stained with the Mallory-Heidenhain stain.

The medial change described above is known in the literature as medial fibrosis. In 1913 Ssobolew described fibrosis of the media in the aorta and iliac artery which he called "cirrhosis of the vessel wall." Staemmler studied the fibrosis of the renal and splenic arteries in relation to the age of the individual. He found an increase in the connective tissue of the media in the third decade. With increasing age the amount of connective tissue in the media becomes more marked. Dietrich found an increased amount of collagen with increased age. Troitzkaja-Andreewa found that the collagenous fibers not only become larger but more numerous. He noted that the precollagenous fibers were transformed into collagen and in advanced age the coarse bundles of collagen lose their sharp

TABLE I

*Cases Without Hypertension. The Degree of Fibrosis of the Media is Estimated*

Case No	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	yrs.			gm.		
1	0	F	?	?	10	Asphyxia
2	14	F	?	225	30	German measles
3	18	F	140/100	275	30	Uremia, chronic nephritis
4	19	M	?	200	30	Miliary tuberculosis
5	21	M	?	375	20	Trauma
6	23	F	?	300	20	Septic abortion
7	24	M	?	365	40	Fracture of neck
8	27	F	112/74	280	30	Leukemia
9	28	M	?	305	20	Trauma
10	29	F	110/80	250	60	Pneumonia
11	29	M	?	355	30	Trauma
12	29	M	110/60	250	20	Miliary tuberculosis
13	29	F	114/70	190	40	Toxemia of pregnancy
			170/125			
14	30	M	?	300	60	Trauma
15	31	F	?	350	50	Hemorrhage from peptic ulcer
16	32	F	?	175	20	Lobar pneumonia
17	35	F	?	250	50	Pneumonia
18	36	M	?	425	30	Portal cirrhosis
19	36	M	?	280	30	Lipoid nephrosis
20	37	M	?	260	30	Undetermined
21	38	M	?	300	50	Meningitis
22	38	F	?	650	30	Rheumatic heart disease
23	39	M	130/70	320	50	Hodgkin's disease
24	39	M	122/65	275	50	Lobar pneumonia
25	40	M	?	250	20	Partial intestinal obstruction
26	40	F	?	300	50	Peritonitis
27	41	M	?	400	50	Coronary arteriosclerosis, old valve defect
28	41	M	?	295	30	Pneumonia
29	42	M	?	425	30	Fracture of skull
30	43	M	?	375	30	Phenobarbital poisoning
31	43	M	?	400	30	Pneumonia
32	44	F	?	260	50	Mesenteric thrombosis
33	45	F	?	250	40	Purulent bronchitis
34	45	M	?	275	20	Septicemia
35	45	M	?	310	40	Trauma
36	46	M	?	225	30	Postoperative hemorrhage
37	46	M	132/80	375	30	Pulmonary embolism
38	47	M	?	300	40	Obstructive jaundice
39	47	M	?	425	30	Trauma
40	48	M	?	380	30	Fracture of neck
41	48	F	?	430	30	Pulmonary tuberculosis
42	50	M	?	400	40	Coronary occlusion
43	49	M	?	360	30	Subdural hematoma
44	50	M	80/50	300	80	Pulmonary tuberculosis

TABLE I (continued)

Case No.	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	<i>yrs.</i>			<i>gm.</i>		
45	50	M	?	460	30	Coronary sclerosis
46	51	M	?	300	30	Appendiceal abscess
47	52	M	?	400	50	Trauma
48	52	M	?	275	30	Pancreatitis
49	52	M	?	385	30	Carbon monoxide poisoning
50	53	M	?	450	30	Aortic aneurysm
51	54	M	138/90	375	50	Carcinoma of rectum
52	54	M	?	450	30	Coronary occlusion
53	54	M	?	300	30	Trauma
54	55	M	?	400	20	Coronary occlusion
55	55	M	?	385	70	Coronary thrombosis
56	55	M	?	400	40	Coronary thrombosis
57	55	M	?	410	40	Perforated gastric ulcer
58	55	M	135/60	350	50	Carcinoma of pleura
59	55	M	?	365	50	Coronary sclerosis
60	55	M	125/85	275	20	Lobar pneumonia
61	56	M	?	400	20	Meningitis
62	57	M	?	250	30	Bronchopneumonia
63	57	M	?	338	50	Undetermined
64	59	M	129/88	475	30	Coronary sclerosis
65	60	F	?	400	50	Portal cirrhosis
66	60	M	?	375	30	Acute leukemia
67	60	M	?	350	30	Syphilitic encephalitis?
68	61	F	?	400	50	Carcinoma of stomach
69	61	M	?	360	40	Trauma
70	62	M	?	300	30	Myelitis
71	62	F	?	300	20	Peritonitis
72	62	M	?	350	80	Hemorrhage
73	63	M	116/100	440	50	Trichlorethylene poisoning
74	63	F	?	260	50	Ulcerative enteritis
75	64	M	?	340	30	Trauma
76	64	M	?	225	30	Carcinoma of stomach
77	64	M	?	325	40	Carcinoma of bladder
78	65	M	140/80	400	30	Carcinoma of stomach
79	65	M	?	450	20	Luetic aortitis
80	65	M	?	410	30	Trauma
81	66	M	?	425	30	Carbon monoxide poisoning
82	66	M	?	400	30	Dissecting aneurysm of aorta
83	67	M	130/70	320	40	Coronary sclerosis
84	67	M	?	400	50	Carcinoma of rectum
85	67	F	?	370	50	Amebic dysentery
86	69	M	?	400	40	Pneumonia
87	69	M	?	425	30	Bronchopneumonia
88	70	M	Normal	320	40	Astroblastoma
89	71	M	?	300	30	Laryngitis
90	73	M	?	280	40	Carcinoma of pancreas



TABLE I (*continued*)

Case No.	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	Yrs.			gm.		
91	73	F	?	290	50	Bronchogenic carcinoma
92	73	F	?	340	70	Ruptured appendix
93	73	M	?	450	40	Coronary thrombosis
94	74	F	?	300	50	Peritonitis
95	74	F	148/92	250	20	Cerebral hemorrhage
96	74	M	?	225	70	Trauma
97	75	F	?	250	30	Adenocarcinoma of gall bladder
98	75	F	?	300	30	Generalized arteriosclerosis
99	76	F	?	375	30	Lipoid nephrosis
100	76	F	?	275	40	Bronchopneumonia
101	77	M	?	400	30	Hydronephrosis
102	78	F	?	325	40	Septicemia
103	79	M	?	425	40	Postoperative ileus
104	79	M	?	330	50	Carcinoma of prostate
105	83	M	?	250	60	Hypertrophic pyloric stenosis

outline and stain diffusely. In infants only the reticulum and a few collagenous fibers could be seen in the media. He found a marked increase in collagen in the third decade. By the 40th year there was more collagen than muscle in the media of large arteries. Fibrosis of the arterioles was not described.

*Influence of Age:* In the small arteries and arterioles of pectoral muscle in my series no instance of marked fibrosis of the media was encountered in an individual under 29 years of age. In one stillborn infant the arterioles contained no fibrous tissue other than the internal elastic lamina and a very fine reticulum surrounding each muscle fiber. Figures 1 and 3 show minimal degrees of fibrosis. After the age of 29 years marked degrees of fibrosis of the media were seen with increasing frequency (see tables).

In Table I the non-hypertensive cases are listed with age, sex, heart weight, the blood pressure when recorded, cause of death, and an estimate in per cent of the amount of blue staining material in the vessel wall when stained with Mallory-Heidenhain stain. The percentage recorded in the tables is an average of estimates of the amount of fibrous tissue in several vessels of the muscle. In Table II the cases of hypertension are similarly tabulated.

TABLE II

*Cases With Hypertension. The Degree of Fibrosis of the Media is Estimated*

Case No.	Age	Sex	Blood pressure	Heart weight	Percentage of fibrosis of wall	Cause of death
	yrs.			gm.		
106	34	F	260/156	470	80	Heart failure
107	36	M	160/106	465	50	Hemorrhage
108	40	F	204/?	400	20	Cerebral hemorrhage
109	43	M	160-240/120	770	40	Heart failure
110	48	F	270/170	682	80	Cerebral hemorrhage
111	49	F	?	575	40	Dissecting aneurysm of aorta
112	51	M	240/?	550	50	Spontaneous rupture of aorta
113	51	F	150/90	275	30	Peritonitis
114	51	M	200/110	450	80	Acute fulminant hypertension
115	54	M	?	575	30	Coronary sclerosis
116	54	M	180/120	650	40	Cerebral hemorrhage?
117	55	M	?	565	50	Uremia
118	55	M	?	575	40	Trauma
119	57	M	170/110	275	40	Congenital cystic kidneys
120	56	F	230/100	350	60	Undetermined
121	58	M	?	580	50	Trauma
122	61	M	?	650	30	Coronary occlusion
123	62	F	155/60	300	30	Peritonitis
124	65	M	?	610	50	Heart failure
125	65	M	?	500	40	Trauma
126	65	M	?	550	30	Trauma
127	65	M	210/100	630	40	Cerebral hemorrhage
128	65	M	?	600	30	Coronary thrombosis
129	70	F	210/90	380	60	Pulmonary embolism
130	70	F	?	450	40	Coronary sclerosis
131	72	F	?	440	40	Undetermined, hypertension
132	73	M	160/80	430	50	Cerebral infarction
133	75	M	160/75	400	30	Bronchopneumonia
134	80	M	190-230/?	400	60	Arteriosclerosis
135	81	M	?	550	30	Pneumonia
136	83	F	170/100	525	50	Cerebral artery thrombosis
137	84	M	?	560	30	Trauma

Figures 2 and 4 show an increase in the degree of fibrosis of the media. A number of cases reached advanced age with only a slight amount of fibrosis. The most marked degree of fibrosis was seen in a 51 year old man who died of acute fulminant hypertension with renal insufficiency. Over 80 per cent of the walls of the arterioles was replaced by fibrous tissue. Only two instances of marked fibrosis were encountered in persons less than 50 years of age. These were

TABLE III

*Averaged Estimated Percentage of Fibrous Tissue in Walls of Small Arteries and Arterioles*

*Cases Without Hypertension*

1st decade	10 per cent	1 case
2nd "	30 "	3 "
3rd "	30 "	9 "
4th "	40 "	11 "
5th "	35 "	18 "
6th "	40 "	22 "
7th "	40 "	23 "
8th "	40 "	17 "
9th "	60 "	1 "
<hr/>		
Average	38 per cent	105 cases

*Cases With Hypertension*

4th decade	65 per cent	2 cases
5th "	45 "	4 "
6th "	47 "	10 "
7th "	35 "	7 "
8th "	45 "	5 "
9th "	40 "	4 "
<hr/>		
Average	45 per cent	32 cases

both cases of severe hypertension where the individual died with cerebral hemorrhage. Figure 2 shows one of the most marked degrees of fibrosis encountered. However, several cases of hypertension in persons of advanced age showed only slight amounts of fibrosis.

Patients with hypertension have somewhat more fibrosis in the small arteries than do non-hypertensives of the same age group. Table III gives the average percentage of fibrosis in the two groups in the decades of life in which hypertension occurred in this series.

The walls of the small arteries and arterioles that contained relatively large amounts of fibrous tissue appeared to be of about the same thickness as the walls of vessels that contained only small amounts of fibrous tissue. With increase in fibrous tissue there is a loss of muscle. The degree of fibrosis is more or less constant in all the arteries that have a diameter less than 150 microns in the same piece of muscle. The small and large arterioles are involved to about the same degree.

The fibers probably do not arise from the fibrocytes of the ad-

ventitia, as Ssobolew suggested, for no nuclei other than those of the muscle may be seen in the media. They probably arise from the reticulum and the fine collagenous fibers of the media.

Fibrosis of an artery may change its microscopic appearance by decreasing the amount of postmortem contraction. Hesse measured the length of the artery in the arm *in situ* and again after it had been removed from the body. In young persons it contracted as much as 40 per cent in length. In other cases, particularly in older persons, it contracted slightly or not at all.

The treatment of the tissue may also affect the appearance of the arteries. In 1908 MacWilliam and Mackie found that it was possible to stimulate and cause contraction of arteries as much as 30 hours after removal from the body. In lower animals this irritability may last for as long as 4 days. This could be abolished by various methods. Depending on the state of portions of the same artery at the time of fixation, sections showed differences of as much as 25 to 100 per cent in the size of the lumen and as much as 30 per cent in the thickness of the media.

In 1934 Moritz attempted to determine whether or not variations in the technical procedures involved in fixing, dehydrating, and embedding tissues might account for variations in the relative thickness of vessel walls. He found that no significant alterations in the ratios of internal to external diameters could be related to variations in laboratory methods. To learn whether the state of contraction of the vessels at the time of removal from the body was rendered permanent by fixation, samples of skeletal muscles were obtained after administration of vasoconstricting and vasodilating drugs. Neither vasoconstriction nor vasodilatation persisted through the process of preparation of the tissues for microscopic examination. He then concluded that observed differences must be regarded as significant. In a later communication the same author with Oldt reported the histological study of the walls and measurements of the internal and external diameters of over 10,000 arterioles and small arteries from skeletal muscle and the gastro-intestinal tracts of 38 control and 38 hypertensive individuals. Thickening of the walls was characteristic of the hypertensives as a group, but these dimensional changes were not great enough in samples of 75 vessels to permit distinction between hypertensives and controls in 80 per cent of the cases. They stated that they were, however, able to recognize cases

of hypertension and controls as such by the presence or absence of arteriosclerosis. They interpreted smooth muscle hyperplasia and degenerative changes in the media as arteriosclerosis. The process not only affected different arteries in the same tissue differently, but in serial sections it was found that different segments of the same artery were affected differently and gave varying wall to lumen ratios.

It should be remembered that a state of contraction of the arteriole may make the vessel appear to have a thickened wall. A rough idea of the degree of contraction may be gained by noting the amount of wrinkling of the internal elastic lamina. By applying varying amounts of tension on the walls of aortas Zon found that the internal elastic membrane became straight at pressures less than diastolic blood pressure. With the application of more weight the elastic membrane stretched like a rubber band.

The wall to lumen ratios of many small arteries and arterioles were determined. There is a correlation between the degree of contraction of the vessel and the wall to lumen ratio. There is a higher wall to lumen ratio in those vessels in which the internal elastic lamina is tortuous. The ratios were so obviously influenced by the state of contraction of the vessel, as seen by the length of the internal elastic lamina, that attempts to measure the vessels accurately were abandoned. The relation between the state of contraction of the artery and the wall to lumen ratio was the only constant relation observed.

Figure 4 shows two arterioles in the same section of pectoral muscle. In one the internal elastic lamina is thrown into folds and the vessel appears to have a thick wall. The elastic lamina of the other arteriole is fairly regular. This vessel appears to have a thin wall. Because of the manner in which the adventitia of many of the arterioles of pectoral muscle blends with the surrounding connective tissue, accurate measurements of the outer margins may be difficult or impossible. Figures 3 and 4 illustrate this source of error.

In the same section of muscle arterioles may show different degrees of contraction and wide variations in wall to lumen ratios. Unless the amount of contraction can be judged, the relative thickness of the vessel wall cannot be determined.

## SUMMARY AND CONCLUSIONS

In a study of specimens of pectoral muscle from 137 individuals a marked variation in the degree of fibrosis of the media was observed. This may be seen in sections stained with the Mallory-Heidenhain stain.

The degree of fibrosis was most marked in cases of severe hypertension. There was a somewhat greater average amount of fibrous tissue in the walls of the arterioles in hypertensives than in non-hypertensives of the same age group. All cases of hypertension did not show marked fibrosis.

No cases of marked fibrosis were seen before the 29th year of life. After that age it was encountered with increasing frequency. However, many persons of advanced age show only minimal degrees of fibrosis.

The connective tissue fibers apparently arise from the reticulum and collagenous fibers in the media.

The wall to lumen ratio is determined by the contraction of the vessel which can be estimated by the length and tortuosity of the internal elastic lamina.

No intimal disease was found in the small arteries or arterioles of pectoral muscle at any age. It was not seen in any case of hypertension.

It was not possible to distinguish between hypertensive and control patients by examination of the small arteries and arterioles of pectoral muscle.

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## DESCRIPTION OF PLATE

### PLATE 119

FIG. 1. Case 116. Male, 54 years of age. Blood pressure 180/120. Heart weight 650 gm. Left ventricular hypertrophy. Death due to cerebral hemorrhage (?).

Arteriole with an outside diameter of 32 microns. A fine layer of reticulum surrounds each smooth muscle cell. Low degree of fibrosis. Mallory-Heidenhain stain.  $\times 600$ .

FIG. 2. Case 106. Female, 34 years of age. Hypertension discovered 1 year before death. Blood pressure 260/156. Death due to acute heart failure. Heart weight 470 gm.

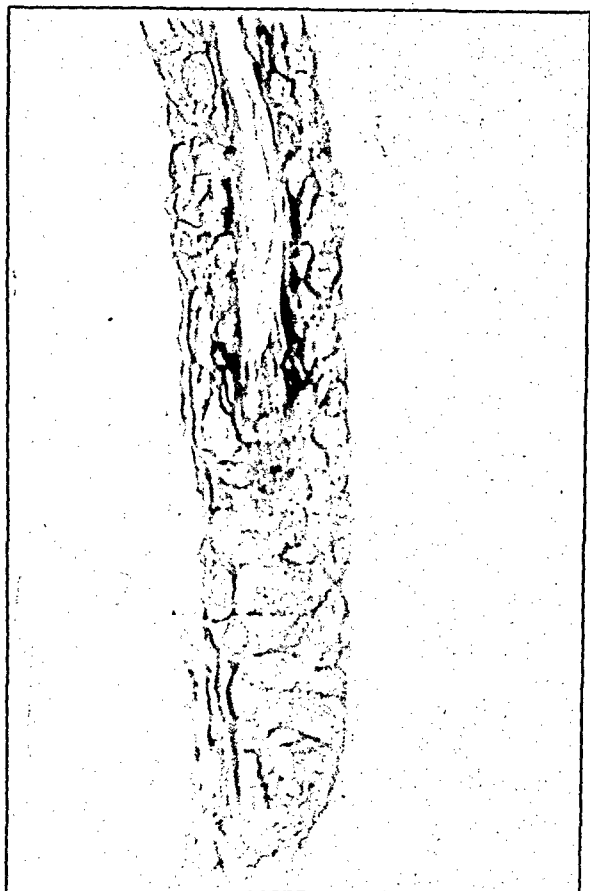
Arteriole with an outside diameter of 78 microns. There is marked fibrosis of the vessel wall. The persistent muscle fibers are seen between the collagenous fibers. Mallory-Heidenhain stain.  $\times 600$ .

FIG. 3. Case 135. Male, 81 years of age. Heart weight 550 gm. Left ventricular hypertrophy. Blood pressure not known. Diabetes mellitus and lobar pneumonia.

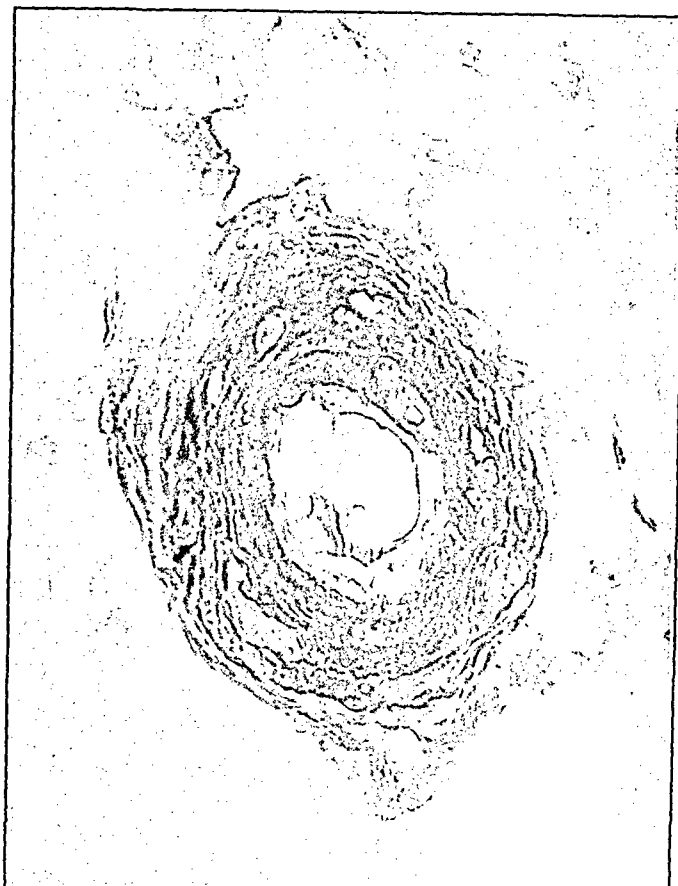
Arteriole with an outside diameter of 40 microns in a relaxed state. Note absence of tortuosity of internal elastic lamina. Wall to lumen ratio 1.0:5.0. Minimal degree of fibrosis. Mallory-Heidenhain stain.  $\times 900$ .

FIG. 4. Case 77. Male, 64 years of age. Heart weight 325 gm. Death from carcinoma of urinary bladder.

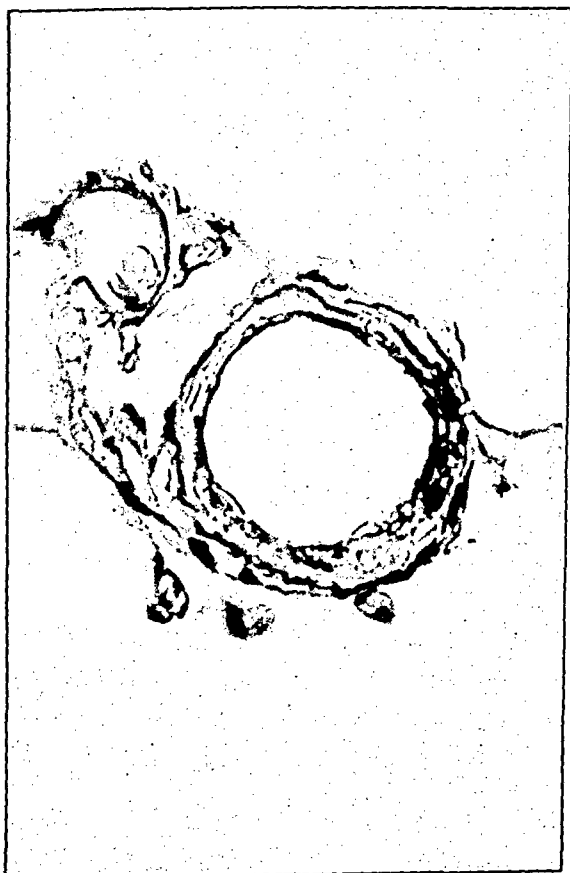
Two arterioles from the same section of pectoral muscle with outside diameters of 85 and 82 microns respectively. The walls of the two vessels are in different states of contraction. The artery in the lower right corner is contracted as is shown by its wavy internal elastic lamina. Its wall to lumen ratio is 1.0:2.0. The artery in the upper left corner is relaxed as is shown by its straight internal elastic lamina. Its wall to lumen ratio is 1.0:4.0. Mallory-Heidenhain stain.  $\times 180$ .



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Structure of Arteries and Arterioles



# MICROTECHNICAL DEMONSTRATION OF IRON\*

## A CRITICISM OF ITS METHODS

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Iron contained in the tissues occurs in two forms. In one group of compounds, generally included under the denomination "hemosiderin," the iron reacts similarly to any inorganic, insoluble iron compound, readily demonstrable by the well known iron reactions of analytic chemistry. In the second group of compounds iron can be demonstrated only after thorough chemical destruction, *e.g.* incineration. In the latter group belong, among others, hemoglobin, methemoglobin and hematin. In this paper I wish to deal with the demonstration of hemosiderin-iron only.

There are three methods and their modifications, respectively, known for the demonstration of iron in microscopic preparations: (1) the Berlin blue method of Perls; (2) the iron sulphide reaction of Quincke (described by Mayer as early as 1850); and (3) the Turnbull blue method of Tirmann and Schmelzer. This last method is based on Quincke's reaction. According to the Berlin blue method of Perls the microscopic section is treated with hydrochloric acid and potassium ferrocyanide. Iron dissolved from hemosiderin by the hydrochloric acid reacts with the potassium ferrocyanide, producing a precipitate of Berlin blue. According to the iron sulphide reaction of Quincke, iron compounds are converted by yellow ammonium sulphide into iron sulphide. On account of the energetic reducing properties of this reagent, ferrous sulphide is formed from both ferrous and ferric compounds. Ferrous sulphide is highly sensitive to oxygen and acids, and preparations will deteriorate within a few days in spite of all possible care. Therefore, the Turnbull blue method of Tirmann and Schmelzer transforms ferrous sulphide by means of an acidulated solution of potassium ferricyanide into Turnbull's blue.

The question as to which of the methods mentioned is the best to

\* Received for publication February 12, 1936.

demonstrate the greatest amount of iron present and to give the truest picture has been rather extensively debated. Generally speaking there are but a few adherents of the Berlin blue method (Zaleski, Arnold and Sumita). According to Sumita no method is superior to that of the Berlin blue. He reproduces a table of Neumann's showing that colorimetric demonstration of ferri-iron as Berlin blue is about 250 times more sensitive than its demonstration as iron sulphide. On the contrary, the great majority of workers on this topic are of the opinion that the iron sulphide reaction and Turnbull's blue method are more sensitive, demonstrating a greater amount of iron than the Berlin blue method. According to Quincke, the details, as revealed by the iron sulphide reaction, are much finer than those by the Berlin blue method. In his opinion this is due to the fact that iron is present in the tissues partly in the form of ferrous compounds which do not react with potassium ferrocyanide. Moreover, he supports the greater sensitivity of the iron sulphide reaction with the observation that egg yolk is stained dark green by ammonium sulphide, whereas if treated with hydrochloric acid and potassium ferrocyanide it becomes but pale blue. He also contends that the Berlin blue method will occasionally produce false positive reactions in the absence of iron compounds. According to Hueck, Turnbull's blue method is more reliable, especially if small amounts of iron are to be demonstrated. He is unable to explain this, as he never could demonstrate ferrous compounds in the tissues with the direct Turnbull's blue reaction. He observed that potassium sulphocyanate demonstrates a conspicuously smaller number of granules than ammonium sulphide. Nishimura's modification of the Tirmann-Schmelzer method consists of employing potassium ferrocyanide instead of ferricyanide after previous treatment with ammonium sulphide by reason of quick oxidation of the white ferro-ferrocyanide formed at first into ferri-ferrocyanide (Berlin blue) by the oxygen of the air. He declares that he was often able to prove the presence of ferrous in addition to ferric compounds in tissue sections. In his opinion the alleged higher sensitivity of Turnbull's blue method is due partly to the fact that it demonstrates both ferric and ferrous compounds. Mallory, too, prefers modifications of Turnbull's blue to the Berlin blue method, partly on the basis of the theory just mentioned, partly because Berlin blue is soluble in an excess of potassium ferrocyanide, causing blurring of the picture. Lubarsch also recommends Turn-

bull's blue method as the most sensitive of all. In short, according to the generally accepted opinion in the medical literature on this question, Turnbull's blue is the most dependable, demonstrating the greatest amount of iron: this tenet is accepted by all textbooks of microtechnique.

In the course of the year I performed the iron reaction on a great number of microscopic preparations and made observations contrary to the general view mentioned. Therefore I set out to reinvestigate the entire question of the microtechnical demonstration of iron. My material was rather miscellaneous: formalin and formalin-alcohol fixed organs from cases of pernicious anemia, organized hematomas of various ages, a case of pigmented giant celled xanthoma and the organs of a rabbit dying of phenylhydrazine intoxication. Many microscopic sections were prepared from all tissue blocks and all types of iron demonstration, as found in the literature, were tried on them, together with some methods devised by me. My results were as follows:

1. I was unable to demonstrate even traces of ferrous compounds in any material with the direct Turnbull's blue reaction. Therefore I see no reason why the Stoeltzner method, using a mixture of potassium ferro- and ferricyanide in order to ensure demonstration of both ferric and ferrous compounds, should ever be employed.

2. Different modifications of the Berlin blue method are by no means of the same value. Those modifications using hydrochloric acid and potassium ferrocyanide separately will invariably show a smaller amount of iron than those using them simultaneously. Those using acid first (method of Stieda) are fundamentally wrong, since the iron dissolved by the acid before the application of potassium ferrocyanide is irretrievably lost. But even those using the reagents in reverse order are objectionable. This is but natural. Ferric chloride is but slowly split from hemosiderin by hydrochloric acid; in the meantime almost all the potassium ferrocyanide taken up by the section during previous treatment with this substance will diffuse away and become so highly diluted in the excess of hydrochloric acid that its concentration will become entirely insufficient. This applies even to the method of Sumita (incubating the sections with a concentrated solution of potassium ferrocyanide). In this way both theoretically and practically only those methods are justifiable that use both reagents simultaneously. But even in the

latter case too short a time of exposure may constitute a serious source of error. Even when using a 10 per cent solution of hydrochloric acid (commercial pure concentrated hydrochloric acid, concentrated about 40 per cent of hydrochloric acid gas by weight, being taken here for 100 per cent) it can be easily observed that for about 20 to 25 minutes the number of blue granules will steadily increase, whereas after 25 minutes no more new granules are noticed. If less concentrated solutions are used the completion of the reaction, of course, will take an even longer time. This has been observed also by Nishimura. The concentration of the potassium ferrocyanide seems to be less important: a 1 to 2 per cent solution is quite sufficient. However, in order to prevent the Berlin blue precipitate from dissolving it is necessary to use a more concentrated solution (this point will be dealt with once more under Step 3). On the basis of experience I suggest the use of a mixture made up of equal parts of a 10 per cent potassium ferrocyanide and a 20 per cent hydrochloric acid solution, prepared freshly with distilled water and filtered. The time of exposure should be 30 minutes. This mixture has a satisfactory stability; it begins to show a greenish tinge after about 45 minutes, but no precipitate is formed even after several hours. Even so, it is advisable to place the slide with the section side down in order to avoid the deposition of any possible precipitate. Berlin blue will not be damaged by the acid as it is almost insoluble in dilute acids; by alkalis, however, it will be bleached. Therefore, counterstaining with lithium carmine is not advisable. For nuclear staining I used Kernechtrot (a red nuclear stain obtained from the German firm, Hollborn), with invariably satisfactory results. The method outlined yields an extraordinarily sharp and complete iron reaction, equal to the iron sulphide but without its drawbacks and decidedly superior to the Tirmann-Schmelzer method and any of its modifications. This was especially conspicuous in cases where minute amounts of iron had to be demonstrated. I did not observe a single case in which Turnbull's blue method gave a critically acceptable evidence of a greater amount of iron than the method described.

3. That in spite of the facts mentioned Turnbull's blue is considered the most reliable method by the majority of workers on this problem is due to several reasons, no one of which, however, can stand objective criticism. First, there are theoretical assumptions



which cannot be substantiated in practice, *e.g.* the presence of ferrous compounds. Hueck's statement that a conspicuously small number of granules react with potassium sulphocyanate is by no means a proof since iron sulphocyanate is easily soluble and its rapid diffusion renders its exact observation impossible. It is interesting that Nishimura, who also uses potassium ferrocyanide when performing the Tirmann-Schmelzer reaction on the basis of the rapid transformation of white ferro-ferrocyanide into Berlin blue, accepts as a possible cause of the alleged lower sensitivity of the Berlin blue method the fact that it fails to demonstrate both ferric and ferrous compounds; the obvious contradiction between his two statements seems to have escaped his attention. Moreover, as has been shown, hemosiderin does not contain ferrous compounds. In the egg yolk test of Quincke the darker color produced with the iron sulphide reaction is not a proof of higher sensitivity but is due simply to the fact that the staining power of ferrous sulphide is greater than that of Berlin blue. This can easily be shown by the following simple test: put one drop each of ammonium sulphide and of a mixture of sodium ferrocyanide and hydrochloric acid on a blotting paper moistened with a highly diluted (0.1 per cent) solution of ferrous sulphate: the spot caused by the first reagent will be dark green, almost black, whereas that caused by the second reagent will be much lighter blue, although both reactions may be considered quantitative. The objection of Mallory to the Berlin blue method is that Berlin blue is soluble in an excess of potassium ferrocyanide, which causes a diffuseness of the staining. I at once state that I never observed blurring attributable to Berlin blue itself; moreover, it can be easily shown that the assertion of Mallory does not hold. Prepare an aqueous suspension of Berlin blue, divide it into four equal parts, dilute these portions to equal volumes with (1) distilled water, (2) 10 per cent hydrochloric acid, (3) a 5 per cent solution of potassium ferrocyanide, and (4) a mixture of the two last solutions. Centrifuge for several minutes and observe the supernatant fluids. In (1) it will be dark blue, in (2) pale blue, and in (3) and (4) pale greenish yellow. This shows clearly that potassium ferrocyanide strongly reduces the solubility of Berlin blue. If the Berlin blue and Turnbull's blue methods are performed on microscopic preparations of the same material, the preparations treated according to Turnbull's blue method often will look without question more blue. If the sections

are compared under the microscope it will be noticed that with the Berlin blue the blue granules are tiny, dense, homogeneous and sharply outlined, similar to the granules of eosinophilic leukocytes. No other morphological element but round granules can be seen. On the contrary, in sections treated according to Turnbull's blue method the blue granules are of a much larger size (this has been noticed by Tirmann, who erroneously attributed this phenomenon to the greater sensitivity of his method); very often they have darker outlines and a paler center; not rarely they are surrounded by a more or less extensive pale blue halo. In addition, from larger granules very often bizarre, whorly or twig-like processes (also with darker outlines) take their origin. These peculiarities observed with Turnbull's blue method are the more marked the more acid is used. If the mixture suggested by the originators (equal parts of 1 per cent hydrochloric acid and a 20 per cent potassium ferricyanide solution) is used, they are quite conspicuous, but are much less so if only one drop of hydrochloric or, even better, acetic acid is added to each 50 cc. of the ferricyanide solution. The section, however, does not become blue as rapidly. If both sections are compared with a third one, made according to the method of Quincke (in which a 1 hour exposure to ammonium sulphide has proved amply sufficient), it will be noticed that the morphology of the iron, according to the Berlin blue method and the iron sulphide reaction, is absolutely identical as regards the number as well as size and shape of the granules; whereas the formations observed with Turnbull's blue are not duplicated by any other method. The cause of this interesting fact is the following: with the Berlin blue method ferric chloride is but slowly split from hemosiderin and bound at once, locally, by potassium ferrocyanide. On the contrary, in the case of Turnbull's blue method, hydrochloric acid dissolves iron sulphide almost with the vehemence of an explosion, but anyhow at a rate greatly surpassing the reaction speed of the formation of Turnbull's blue, the latter being retarded also by a semipermeable membrane of its own substance formed on the surface of the granules. In addition, particles are torn and carried off by gas bubbles. The curious artifacts described are formed in this way, as can easily be observed if the conversion in sections of ferrous sulphide into Turnbull's blue is examined under the microscope. Of course, if, following the suggestion of Mallory, no acid is used these faults will not occur, but the transformation of

ferrous sulphide into Turnbull's blue will be an incomplete one, and in addition many granules will acquire a dingy greenish color, rapidly fading out under the coverslip. Another method devised by Mallory, using 5 per cent acetic acid, is in no respect superior to the original one. Should someone, convinced of the superiority of the iron sulphide reaction, prefer the same, I would advise him to render his preparations durable, not by the Turnbull blue method, but rather by converting ferrous sulphide into either copper or lead sulphide. This can easily be accomplished by washing the section (treated previously with ammonium sulphide) first with pure diluted ammonia, then with distilled water and placing it for 1 hour in either a 5 per cent copper sulphate or lead nitrate solution. The deep black granules of copper or lead sulphide will give in sections counterstained with methylene blue or any red nuclear stain a beautiful picture. Hematoxylin is not recommended for counterstaining. The granules will be but slightly larger than those of the original ferrous sulphide, the difference being insignificant.

While working on this subject I was much annoyed by the fact that both the Berlin blue and Turnbull's blue preparations soon began to lose their color and within a few months, or even weeks, became entirely unsuitable for comparison. This phenomenon is well known and was described by Gans in 1923. Although the preparations can be restored by removing the coverslip, I tried to find a method by which the sections could be made permanent. My experiments were based on the following idea: fading out of Berlin blue or Turnbull's blue sections is quite different in nature from that of hematoxylin or methylene blue. In a section stained with hematoxylin, fading starts from the margins and is progressive toward the center; the process is irreversible. It is probably caused by oxidation. Decolorization of the methylene blue stain can be almost completely prevented by the use of acid-free balsam; therefore it is to be attributed to an acid reaction. Fading out of an iron reaction invariably starts at the center and if the section is not much smaller than the coverslip, its peripheral parts will not lose their color. The decolorization cannot be retarded by using neutral balsam. These facts, together with the quick restoration of the preparations if exposed to air or, even better, to a diluted hydrogen peroxide solution, point to the decolorization being due to reduction. Canada balsam, too, like resins in general, probably takes up oxygen while

drying, depriving the section of its oxygen in this way and reducing both Berlin blue and Turnbull's blue to colorless ferro-ferrocyanide. On this basis it seemed that a mounting medium rich in oxygen would prevent or at least retard the fading out of sections. This idea proved to be sound. I diluted inspissated, almost dry Canada balsam with old, resinified oil of turpentine (known to contain peroxides) until it became of syrup-like consistence and tried to mount sections with it. It turned out that all sections mounted with this medium were much more durable than those mounted with simple Canada balsam. On account of the shortness of time that had elapsed since my experiments (5 months) I am unable to state the exact duration of sections preserved in this way. At any rate, all my preparations mounted with Canada balsam almost completely faded out in this time, whereas those mounted with the new medium remain unchanged. Canada balsam preparations all faded out within less than 2 days if kept at  $56^{\circ}\text{C}$ ., whereas those preserved with oil of turpentine completely withstood this temperature for more than 14 days. Oil of turpentine does not destroy either carmine or Kernechtrot stain. I tried also to dilute Canada balsam with a solution of benzoyl peroxide in xylene; this method, however, proved to be much inferior to the first one. Therefore, I recommend the mounting of iron reaction preparations with Canada balsam diluted with old, resinified oil of turpentine.

#### SUMMARY AND CONCLUSIONS

1. Hemosiderin does not contain ferrous compounds demonstrable with the direct Turnbull's blue reaction.
2. The alleged superiority of the Tirmann-Schmelzer modification of Turnbull's blue method is based partly on erroneous theoretical conceptions and partly on the misinterpretation of artifacts.
3. The best microtechnical reagent for iron is a mixture of equal parts of 20 per cent hydrochloric acid and a 10 per cent solution of potassium ferrocyanide. The exposure should be 30 minutes. Results of equal quality are produced by converting ferrous sulphide obtained in Quincke's reaction into copper or lead sulphide.
4. Both Berlin blue and Turnbull's blue preparations can be made durable by diluting the Canada balsam to be used in mounting sections with old, oxidized oil of turpentine.

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## DESCRIPTION OF PLATE

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### PLATE 120

All photomicrographs were made of the same material (pigmented giant celled xanthoma of a tendon sheath) under identical optical conditions. Hematoxylin nuclear stain.

FIG. 1. Author's modification of the Berlin blue method.  $\times 300$ .

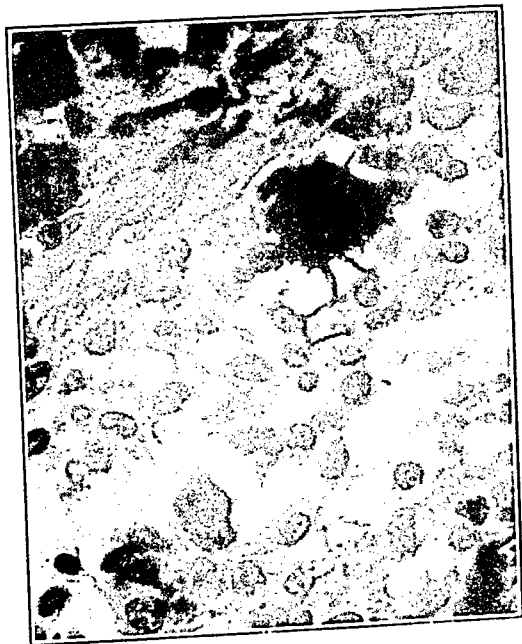
FIG. 2. Author's modification of the Tirmann-Schmelzer method (conversion of ferrous sulphide into lead sulphide).  $\times 300$ .

FIG. 3. Turnbull's blue method. At "A" granules appear as tiny ringlets (diffusion artifact).  $\times 300$ .

FIG. 4. Typical artifact (twig-like processes) as produced with the Turnbull's blue method.  $\times 300$ .



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Microtechnical Demonstration of Iron





# THE REGENERATION OF AUTOPLASTIC SPLENIC TRANSPLANTS \*

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The first successful autotransplantation of splenic tissue in animals was made by Marine and Manley.<sup>1</sup> They found that subcutaneous or intramuscular splenic transplants in the rabbit were successful in almost 100 per cent of instances, and that immaturity of the animal was a powerful stimulus to the growth of the transplants. In immature rabbits with one-eighth of the spleen removed there was a slight growth of the transplant which increased rapidly following subsequent total splenectomy. They believed that this stimulus to growth was chemical in nature, because the grafts were removed from their normal neurovascular supply. The histology of the transplants was identical in all characteristics with normal spleen tissue. Spleen grafts, if made in immature rabbits accompanied by complete splenectomy, were permanent throughout the life of the animal. They further observed that although spleen transplants in rabbits were successful, in the absence of a physiological deficiency of spleen tissue they soon atrophied. When there was a definite splenic deficiency due to total splenectomy, autotransplants were not only successful but they grew and increased in size. In mature rabbits, whether splenectomized or not, the transplants regenerated but were slowly resorbed. From their observations they concluded that in the rabbit the spleen is most important in early life. After sexual maturity it is either unimportant or its function may readily be assumed by other hematopoietic tissues.

Subsequently Perla and Marmorston-Gottesman observed the regeneration of autoplasmic splenic transplants in the rat.<sup>2,3</sup> They found that such transplants in the adult rat regenerated and grew in over 90 per cent of instances, even in the presence of the spleen. In many instances it was further observed that the regenerated transplants may function in place of the normal spleen. As evidence of this it was found that in a strain of rats that developed *Bartonella muris* anemia after splenectomy, autoplasmic splenic transplants, performed 7 weeks prior to splenectomy, afforded protection against

\* Received for publication February 10, 1936.

this disease in more than 50 per cent of instances. A conspicuous difference was noted in the histology of the transplants that protected and those that did not protect the animal. In the protected rats the red pulp appeared normal and showed a large amount of phagocytosis of iron-containing pigment by the reticular cells and macrophages. In the transplants of the animals that died of *Bartonella muris* anemia only a few lymphoid cells were found in the red pulp. Many of the nuclei of the reticular cells showed evidence of necrosis. Only minute amounts of iron-containing pigment were found in the macrophages. The sinuses of the red pulp were engorged with blood. The follicles were sharply defined and showed no pathological change.

In the present communication the detailed histogenesis of autoplasmic splenic transplants is reported.

#### METHODS \*

In this work 24 mature albino rats free from *Bartonella muris* infection (Wistar stock), about 3 months of age, were used. The spleen was exposed through a small incision in the abdominal wall and one pole was gently pulled up into the opening. A piece was cut off for transplantation. Pieces of spleen about 6 mm. in diameter were immediately placed in small prepared pockets in the abdominal wall. Great care was exercised to avoid hemorrhage into these pockets, and if hemorrhage occurred a new pocket was made. The sites of the transplants were indicated by closing the mouth of the pocket with a black silk suture. The method of transplantation was essentially the same as that employed in the transplantation of thymus and lymph node tissue reported in previous communications by Marmorston-Gottesman and Jaffe.<sup>4,5</sup> Four transplants were usually inserted in each animal. Two rats each were killed at intervals of 2, 18, 24 and 36 hours, and 3, 4, 5, 6, 7, 12, 21 days and 7 weeks after operation. The transplants were carefully removed, fixed in Bouin's fluid, and embedded in paraffin. Sections were stained with hematoxylin-eosin, Verhoeff's elastic tissue stain, Pacini's stain, and by Bielschowsky's silver impregnation method.

\* I am indebted to Dr. Jessie Marmorston for surgical assistance in the transplantations.

## PRESENTATION OF DATA

This study is based on an examination of 96 splenic transplants. A description of the successive stages of degeneration, regeneration and growth of the transplants follows.

*2 Hours:* Only at the cut edge of the splenic transplant is there evidence of necrosis in the splenic tissue. In the remaining tissue the cells are intact, the architecture clear. In the muscle surrounding the transplant there is little cellular reaction to its presence. The cytoplasm of the surrounding muscle fibers is swollen and shows granular degeneration.

*15 Hours:* There is evidence of necrosis throughout the entire transplant except for a thin outer zone of viable tissue. This zone shows well formed lymphocytes and reticular cells. The rest of the transplant architecture is poorly defined. The nuclear elements show various stages of karyorrhexis. In some sections extensive hemorrhage is present in and about the transplant. The surrounding tissue contains many polymorphonuclear leukocytes, very few mononuclear phagocytes but a considerable amount of edematous fluid.

*24 Hours:* The transplant is infiltrated with blood. There is an extensive necrosis of the entire splenic tissue except for a narrow zone where a layer of well defined cells is present, at the outer margin of the transplant. Fragments of nuclear elements are present throughout. The surrounding tissue is edematous and is infiltrated with many polymorphonuclear leukocytes and a few mononuclear phagocytes.

*36 Hours:* In some areas a portion of the splenic capsule is still preserved, and just beneath the capsule within the transplant reticular cells, lymphocytes and well formed sinuses are seen. There are small areas of hemorrhage and erythrophagocytosis by macrophages is seen. There is a zone of apparently viable reticular cells at the periphery which merges with the necrotic tissue that occupies the rest of the transplant. In many areas the entire architecture of the splenic tissue is destroyed. Nuclear fragments, cellular debris and many red blood cells and polymorphonuclear leukocytes are present within the transplant. In these areas lymphocytes and reticular and endothelial cells in all stages of degeneration are visible. The

blood vessels are included in the massive necrosis which is seen in the transplant.

The reticulum and collagen fibrils within the area of necrosis are not distinguishable. A few elastic fibers are still present in the connective tissue septa of the transplant. The remnant of capsule around it contains some elastic tissue. That of the small arterioles throughout the transplant is destroyed.

Around the periphery of the transplant the tissue is edematous, and phagocytic macrophages, many of which have engulfed debris and red blood cells, are present in large numbers. Occasional polymorphonuclear leukocytes are seen and a zone of young fibroblasts surrounds the transplant.

*48 Hours:* In the central portions of the transplant the splenic tissue is destroyed. The site of the malpighian corpuscles is discernible but the cells show all stages of degeneration. Throughout most of this area the reticular and endothelial cells are necrotic. In many places they are swollen and show evidence of erythrophagocytosis.

At the periphery of the transplant and beneath the remnant of the capsule numerous reticular-like elements are present. These appear to be growing into the transplant (see Fig. 1). The cytoplasm of these cells forms an irregular syncytium. Red cells are present in ill defined vascular spaces in this zone, but few lymphocytes are seen. The reticulum of the capsule of the transplant is sharply defined and in the tissue surrounding the latter the capillaries are congested, areas of hemorrhage are seen, and many polymorphonuclear leukocytes and phagocytic macrophages containing ingested red blood cells are present.

*3 Days:* In larger transplants the outer half is completely replaced by a mesh of syncytial reticular cells which surrounds and invades the central area of necrotic tissue with finger-like projections.

In one small transplant the reticular cells have regenerated and have almost completely overgrown the transplant. It is completely replaced by what appear to be reticular cells (see Fig. 2). There are few lymphocytes present. The fibrillar and cellular reticulum has formed a mesh of sinuses which gives it a honeycomb appearance. These sinuses are lined with flattened endothelium and

in places it is apparently proliferating and large cells lie free in the sinuses. In other areas several of these cells have fused to form irregular multinuclear giant cells. In reticular and endothelial cells the cytoplasm is scanty and vacuolated. The nuclei are large, pale and vesiculated, and contain finely scattered chromatin material. The cytoplasmic processes anastomose with those of surrounding cells. The fibrillar reticulum can be traced about the sinuses, weaving an interlacing mesh throughout the transplant.

In larger transplants the formation of sinuses has not as yet occurred, although capillaries containing blood cells are present.

*4 Days:* A wide outer zone of the transplant shows large numbers of reticular cells, some of which contain mitotic figures. Their cytoplasm forms a loose syncytial mass penetrating the inner portions of the transplant. Some of these cells contain iron pigment. There are no lymphocytes in this outer zone but there are many red blood cells which lie in irregular, poorly defined sinusoidal spaces. Elliptical endothelial cells, apparently arising from the reticular cells, line the irregular blood spaces in some places. The formation of new fibrillar reticulum forming primitive sinuses within the outer zone can be distinguished. In one area a large mass of reticular cells is arranged in a concentrically lamellated whorl about a capillary space and a few elements resembling small lymphocytes are found in the interstices of this tissue. The reticular fibrils penetrate the outer portion of what appears to be a primitive follicle (see Fig. 3).

In the center of the transplant there is a small area of necrosis, surrounded by a broad zone of mononuclear phagocytes, containing nuclear debris, red blood cells and hemosiderin. Numerous fibroblasts are seen about the transplant and in some areas they seem to invade it. This does not occur in the zones covered by transplanted splenic capsule. The connective tissue of the capsule and the reticulum in the outer zone of the plant is clearly defined. Extending from the capsule are connective tissue septa in the process of formation.

*5 Days:* There is still a central area of necrosis with a zone of hemorrhage. The outer half of the transplant shows complete regeneration. A partly developed follicle close to an arteriole can be seen. All stages of lymphoid cell formation are visible. The reticular cells

vary considerably in size and shape, and transition forms between large, oval reticular cells and fully developed lymphocytes may be distinguished.

The reticular fibrils in the regenerated part of the transplant form a fine pattern of sinuses. In one area a prominent and well formed arteriole lies in the center of a follicle. The elastica of the wall is fully developed.

The connective tissue of the capsule is clearly defined. The connective tissue septa are present, extending into the transplant. Scattered outside of its capsule are reticular cells, lymphocytes, occasional polymorphonuclear leukocytes and mononuclear macrophages containing blood pigment.

*6 Days:* The transplant presents a remarkable picture. There is a small central area of necrosis. Within this area are ghosts of large cells containing blood pigment but showing evidence of degeneration. The necrotic zone merges imperceptibly with a zone of loosely arranged, large mononuclear cells containing large amounts of blood pigment. These large reticular-like elements gradually become more compact as the outer portion of the transplant is approached. An occasional well formed arteriole is seen in this area. The outer two thirds of the transplant show fully regenerated splenic tissue. Sinuses containing red blood cells are present. Reticular and endothelial cells are rich in blood pigment. Lymphocytes, although scattered through the pulp, accumulate more prominently in some areas where follicle formation is apparently occurring. Cells suggesting transition forms between large oval reticular cells and fully developed lymphocytes may be distinguished.

Within a primitive follicle a small arteriole seems to be in the process of formation. The outer portion is composed of whorls of flattened reticular-like cells with elliptical nuclei. The inner portion of the structure consists of a syncytium of large, pale, reticular elements with pale staining cytoplasm. The lumen is not definitely seen. A well defined zone of fibrillar reticulum is discernible in the outer portion of the wall. Another arteriole shows a later stage with more definite lumen formation. The connective tissue of the media is more deeply stained and condensed. The lumen contains red blood cells and is lined with a syncytium of large, vesiculated endothelial cells. The transplant has a well defined capsule which sharply separates it from the surrounding tissue. Surrounding the

transplant, outside of the capsule, is a fibrillar connective tissue structure containing macrophages rich in blood pigment. In one area in direct contiguity with the capsule, but outside of it, there is a large cluster of reticular cells arranged in a concentric whorl showing evidence of follicle formation and containing various transition forms between reticular cells and lymphocytes.

*7 Days:* There is no area of central necrosis. The entire inner two-thirds of the transplant is completely replaced by a loose syncytium of reticular cells. Many of these cells in this zone and in the outer zone contain a large quantity of blood pigment. In the outer third of the transplant, in addition to the syncytial mass of reticular elements, there are several well defined follicles with lymphocyte formation. Cells suggesting all stages of lymphoid cell formation from large reticular cells to lymphocytes are present. An occasional normoblast may be seen. A large arteriole in the process of formation is evident in one area. The lumen is lined by flattened endothelial cells and about this is a loosely arranged whorl of reticular cells.

The sinuses in the outer zone are not so clearly defined as in some of the other transplants, but where they occur they are markedly distended with blood. The capsule is composed of dense collagen fibrils and well defined trabeculae extend from the capsule into the transplant.

*12 Days:* The transplant is replaced by large numbers of reticular cells. Sinuses containing blood elements are distinguishable. In the outer third are scattered lymphocytes. Veins and arterioles are present. In one area close to a newly formed arteriole there is a clump of modified reticular cells which are round and vesiculated, but their nuclei contain more condensed chromatin masses than are found in reticular cells. They appear to be lymphoblastic cells. Cellular elements suggesting gradations from these to adult lymphocytes may be distinguished. Many pigment-laden mononuclear macrophages are present in the transplant. It is demarcated by a definite capsule containing collagen fibers. The surrounding tissue contains a few polymorphonuclear leukocytes and large macrophages laden with iron pigment.

*21 Days:* The transplant shows evidence of complete regeneration with the formation of a capsule and trabeculae. Foci of lymphocytes surround the arterioles and suggest follicle formation. Well formed

sinuses are present and these are congested. There is evidence of erythrophagocytosis by macrophages. Occasional megakaryocytes are seen in the red pulp.

*7 Weeks:* The transplant is a small spleen about 1.5 cm. in diameter. The elements are completely differentiated. The splenic follicles and the blood sinuses, with the normal red pulp elements, including megakaryocytes, are present. Blood pigment is present within the macrophages and reticular and endothelial cells (see Fig. 4).

### DISCUSSION

The phylogenetic and ontogenetic development of the spleen may be simulated in the adult rat in the regeneration of autoplasmic transplants of fragments of splenic tissue within a few days. The observations reported in this communication suggest that the reticular cells of the adult spleen retain their potentiality for differentiation and that they may be the precursor of the structural cellular elements of the spleen. These studies further suggest that the lymphoblastic tissue may arise from the reticular cell.

Phylogenetically it appears that the entire pulp of the spleen (red and white) develops from a common ancestral mesenchymal reticulum. In certain lower vertebrates, as in fish and amphibia, there is no distinction between the malpighian corpuscles and the red pulp. In the selachians, reptiles and birds, the lymphatic follicles become prominent and stand out against the vascular pulp, but masses of lymphocytes are scattered throughout the red pulp (Klemperer<sup>6</sup>). In the human, the spleen arises embryologically in the dorsal mesogastrium from a group of round cells which appear cytologically identical with those of the surrounding mesenchyme but are more compact. During the early phase of its development the undifferentiated mesenchyme increases in amount and forms a rich vascular network. Later the pulp reticulum, venous sinuses, arterial sheaths and lymphatic tissue develop. At 8 weeks the mesenchymal cells are surrounded by delicate fibers (Ono<sup>7</sup>). In the 3rd month a capsule and trabeculae develop. During the 5th month a vascular bed develops by separation, loosening and rearrangement of the reticular cells. The venous sinuses consist of delicate endothelial-lined tubes apparently continuous with the primary embryonal plexus of veins. At this time hematopoiesis is observed in the growing spleen.



Hemocytoblasts separate from the fixed mesenchymal cells. These differentiate into erythroblasts. After the 5th month this process decreases and is practically absent in the 6th month (Ono).

According to Ono, the development of the lymphatic tissue is associated with the formation of the arterial system. When the branches of the splenic artery acquire a muscular wall during the 4th month, they become surrounded by a mantle of reticular tissue. Early in the 5th month within these areas accumulations of lymphoid cells appear which separate themselves from the surrounding reticular cells. The follicles develop during the 5th and 6th month within the angles of the arterial tree. The vascular network of the follicles is fully developed at the end of fetal life.

From the study reported in the present communication it is evident that the histogenesis of the splenic elements in the regeneration of the autoplasmic transplants repeats the ontogenetic development of the spleen and suggests that the major elements of this organ have a common origin in the mesenchymal reticulum.

It is striking that the developmental potentiality of the reticular cell is retained in adult life in the rat and that regeneration of splenic autotransplants readily occurs even in the presence of most of the spleen.

A comparison of the regeneration of thymus autoplasmic transplants<sup>4</sup> and of lymph node transplants<sup>5</sup> with that of splenic tissue reveals the fact that the reticular cell in each case is the common ancestral cell of the structural elements subsequently found in the fully regenerated tissue. Morphologically the reticular cells of the lymph node, thymus and spleen transplants which survive in the extreme outer zone are apparently identical, and are closely related in their capacity to form lymphoid tissue and in the property of phagocytosis. But in each instance they must possess some specific growth trend different from the reticular elements of other organs.

#### SUMMARY AND CONCLUSION

The histogenetic development of the regeneration of autoplasmic splenic transplants was studied in a series of adult albino rats. It was found that the transplanted splenic tissue undergoes rapid degeneration within the first 24 hours. A thin zone of viable reticular cells survives. There is little inflammatory reaction about the

transplant. By the 3rd day there is marked proliferation of the reticular cells at the outer zone and streamers of cells are seen penetrating the necrotic center of the transplant. In some instances the entire transplant is replaced by reticular cells which have formed the pattern of a sinus organ. By the 3rd or 4th day lymphocytes appear irregularly in clusters and apparently all stages of lymphoid cell formation are observed.

By the 6th day the outer two-thirds of the transplant are replaced by reticular elements arranged in an intricate mesh as in the adult spleen, with sinus formation, and ill defined clusters of lymphocytes are present. Complete regeneration of large transplants occurs from the 12th to the 21st day and the morphological structure of adult spleen tissue is apparent, with well developed capsule and trabeculae.

These studies suggest that the reticular cell of the adult spleen retains its potentiality for differentiation and may be the precursor of the structural elements of the spleen.

ADDENDUM: Some months after this work had been completed a report on the behavior of transplanted spleen by Silberberg appeared (*Arch. Path.*, 1935, 20, 216). In autotransplantation of splenic tissue he also observed complete regeneration in 16 to 21 days. He finds the reticular cells the most resistant to necrosis but does not commit himself with reference to the transformation of these cells to lymphoblastic tissue.

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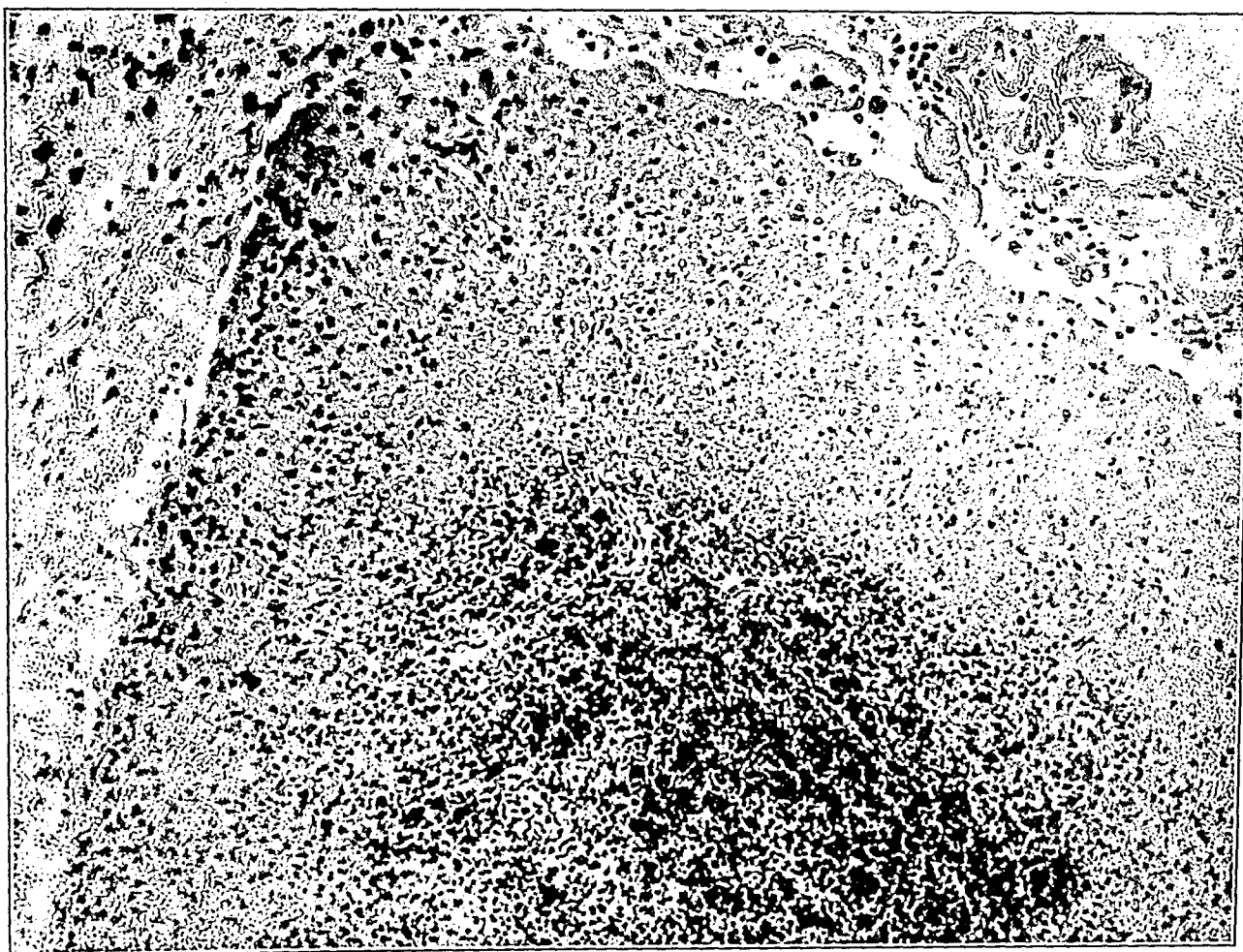
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## DESCRIPTION OF PLATES

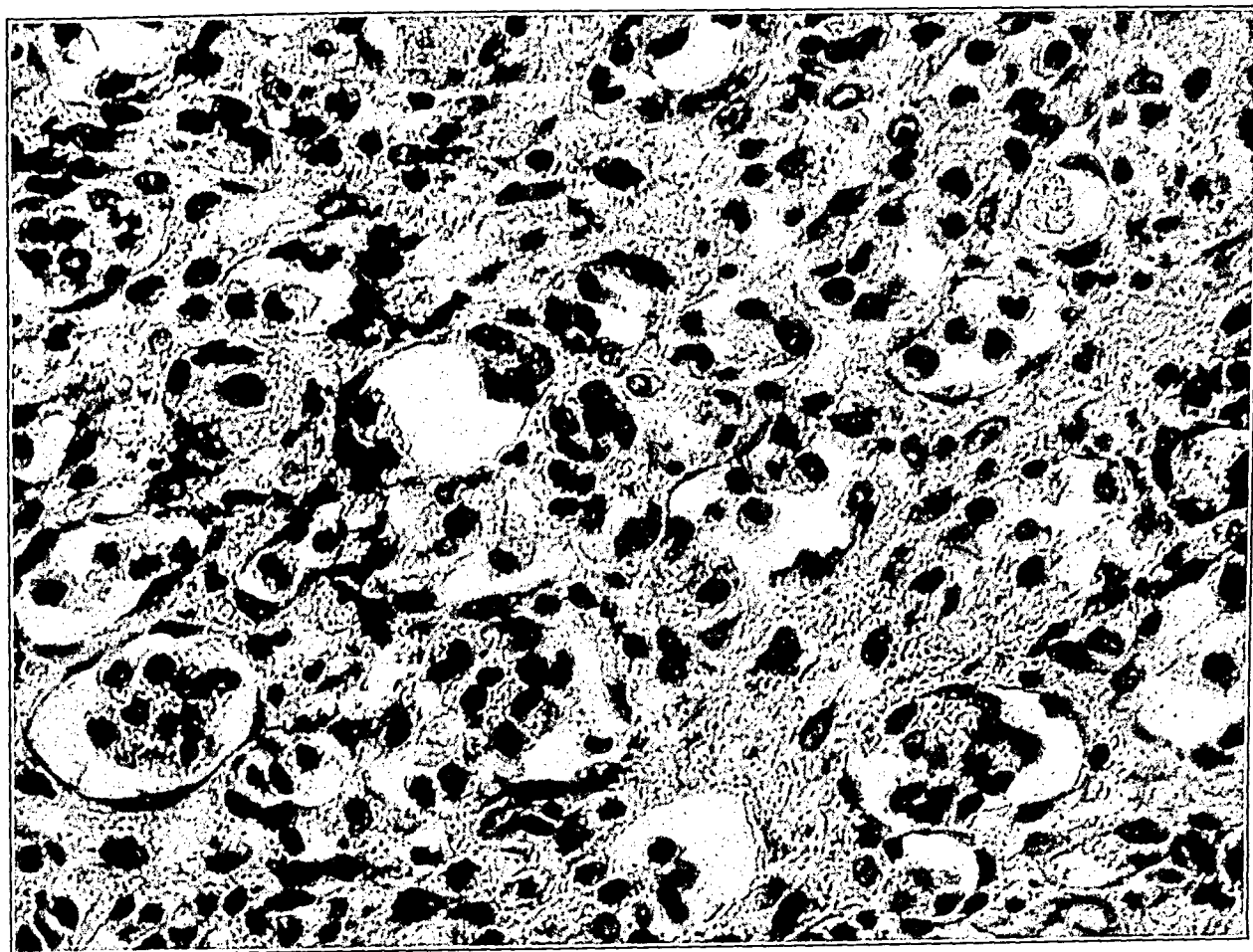
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### PLATE 121

- FIG. 1. Autoplastic splenic transplant, 48 hours. Necrosis of inner portion of the transplant. Outer zone contains numerous reticular cells growing into the transplant and a few scattered lymphocytes. Hematoxylin and eosin stain.  $\times 100$ .
- FIG. 2. Autoplastic splenic transplants, 3 days. Small transplant showing complete replacement by reticular cells and sinuses. Few lymphocytes present. Numerous sinusoidal structures lined with flattened endothelium. In some sinuses endothelial cells are present in the lumens. The reticular cells fill the intersinusoid spaces. Hematoxylin and eosin stain.  $\times 440$ .



1



2

Perla

Regeneration of Autoplastic Splenic Transplants



## MENINGOCOCCUS MYOCARDITIS \*

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Myocarditis due to the meningococcus is apparently an extremely rare disease, judging from the paucity of the relevant literature. With the exception of the reports of Westenhoeffer<sup>1</sup> and Gruber,<sup>2</sup> which are quoted in some of the more comprehensive reference books, case reports of meningococcus myocarditis could not be found. There are a number of instances of meningococcus endocarditis reported in the literature. When these case reports were studied, lesions in the myocardium were encountered which the respective authors merely mentioned and which apparently were not considered to be significant. The purpose of this communication is to stress the occurrence and the clinical and pathological significance of meningococcus myocarditis, to list the changes in the myocardium described in the literature, and to report 2 cases of myocarditis in two patients who died of meningococcus meningitis.

### REVIEW OF LITERATURE

Klebs<sup>3</sup> in 1865 described an enlarged heart associated with acute endocarditis of the mitral valve in a case of epidemic meningitis. The skeletal muscles revealed an increase in connective tissue cells with a few abscesses and some necrosis. He maintained that the myocardium revealed similar changes which, however, were not described.

Warfield and Walker<sup>4</sup> in 1903 reported a case of acute ulcerative endocarditis caused by meningococci. The heart muscle, grossly, was soft and friable, brownish gray, and streaked with white and yellow lines, but was not described histologically.

Westenhoeffer<sup>1</sup> in 1906 found minute leukocytic infiltrations in the myocardium in 3 cases of epidemic meningitis. He described inter- and intramuscular leukocytic infiltrations, some of which were circumscribed and others diffuse.

\* Aided by a grant from the A. Kuppenheimer Fund.  
Received for publication February 28, 1936.

Cecil and Soper<sup>5</sup> in 1911 described a slight increase in connective tissue in the myocardium in a case of meningococcus endocarditis.

Netter and Debré<sup>6</sup> in 1911 stated that heart complications are rare. They maintained, apparently quoting Westenhoeffer, that the myocardium of patients dying from fulminating meningococcus sepsis may show small areas of inflammation caused by meningococci.

Finley and Rhea<sup>7</sup> in 1912 described a "red" myocardium in a case of meningococcus endocarditis, but gave no histological description.

Jochmann,<sup>8</sup> in his textbook on infectious diseases, in 1914 stated that the myocardium often showed intramuscular round cell infiltrations which were partly diffuse and partly circumscribed, and which in some cases might explain death from myocardial failure. However, none of his own observations was given.

Gruber<sup>2</sup> in 1916 stated that of 15 cases of meningococcus meningitis, 8 revealed unquestionable myocarditis, while 7 showed only minute areas of inflammation. The inflammatory lesions could not be interpreted as miliary abscesses. Occasionally the inflammatory cells were perivascular in distribution. In one instance areas of necrosis, fibroblasts and giant cells were encountered, findings that suggested the possibility of a rheumatic infection. None of the sections of the myocardium revealed the presence of microorganisms.

Ghon<sup>3</sup> in 1916 described a case of meningococcus septicemia in which the myocardium showed acute inflammatory changes and many meningococci.

Krumbhaar and Cloud<sup>10</sup> in 1918 reported 3 cases of acute meningococcus endocarditis. A flabby heart was described grossly in only 1 case.

Herrick<sup>11</sup> in 1918 summarized the heart findings in 31 autopsies. A fibrinopurulent pericarditis was found in 4. In 4 others there was a seropurulent pericarditis. Hypertrophy of the heart, dilatation of the heart and acute tricuspid endocarditis were found respectively in 3 other cases. Histological examinations of the myocardium were not reported.

Worster-Drought and Kennedy<sup>12</sup> in 1919 described a large soft heart. The heart was examined only grossly and the diagnosis of myocarditis was made. It was described as follows: "There were mottled, small pale areas in the myocardium alternating with con-



gested areas, and the heart muscle fibers showed a tendency to fall apart."

Kennedy<sup>13</sup> in 1926 described a brown atrophy of the heart in a case of meningococcus septicemia.

Rhoads<sup>14</sup> in 1927 described a vegetative endocarditis due to meningococci. Microscopically a thickened epicardium was infiltrated with lymphocytes. Between the muscle fibers there were many endothelial leukocytes, polymorphonuclear neutrophils, eosinophiles and lymphocytes. A few areas showed necrotic bundles of collagen fibers surrounded by endothelial leukocytes, strongly suggesting Aschoff bodies. In certain areas there was fairly extensive necrosis of muscle fibers with replacement by granulation tissue. Meningococci were not described in the myocardium.

Moleen and Seecof<sup>15</sup> in 1929 described hemorrhages in the endocardium and pericardium in their case of meningococcus septicemia. The heart was soft and flabby.

MacMahon and Burkhardt<sup>16</sup> in 1929 stated that the myocardium in their case of meningococcus endocarditis showed surprisingly few changes. There were a few, small, perivascular foci of polymorphonuclear leukocytes, and occasional necrotic muscle fibers were invaded by endothelial leukocytes.

Baehr and Klemperer<sup>17</sup> in 1929 found multiple foci of necrosis and lymphocytic infiltrations in the myocardium in their fulminating cases of meningococcus bacteremias. Some of the polymorphonuclear leukocytes contained a few Gram-negative diplococci.

Bancker<sup>18</sup> in 1930 noted eosinophilic cells, lymphocytes, endothelial leukocytes and polymorphonuclear leukocytes between the heart muscle fibers in a case of meningococcus endocarditis. There were also a few areas containing necrotic bundles of collagen fibers which were surrounded by endothelial leukocytes, thus resembling Aschoff bodies. Necrosis of muscle fibers with replacement by granulation tissue was also present. His findings were practically similar to those of Rhoads.

Gwyn<sup>19</sup> in 1931 described myocardial changes in a case of subacute meningococcus endocarditis. A few polymorphonuclear leukocytes were found between the heart muscle fibers and there was edema of the connective tissue. Also, some scarring was observed along the vascular channels which indicated the presence of chronic myocarditis. This moderate perivascular fibrosis was diffuse and

in many instances nodular. The picture was suggestive of a rheumatic infection but no Aschoff bodies were seen.

Stevenson<sup>20</sup> in 1931 reported a case of chronic meningococcus septicemia with bacterial endocarditis. He described minute abscesses in the wall of the right ventricle in which Gram-negative intracellular diplococci could be demonstrated in the pus cells. There was also an early necrosis of the muscle fibers.

Lemierre, Laporte, Reilly and Laplane<sup>21</sup> in 1934 described severe changes in the myocardium in a case of meningococcus endocarditis. The normal muscle fibers were replaced by a homogeneous material. Other fibers were separated by proliferation of connective tissue.

Ghon<sup>22</sup> in 1934 in a case of acute insufficiency of the suprarenals in meningococcus septicemia described polymorphonuclear leukocytes in the interstitial tissue surrounding minute blood vessels of the heart muscle. Many Gram-negative intra- and extracellular diplococci were found in the myocardium.

This survey of the literature reveals the apparent rarity of meningococcus myocarditis. As can be seen, observations of isolated cases of meningococcus myocarditis not accompanied by meningococcus endocarditis were reported only 5 times. It is interesting that in 1 of these cases, as well as in 4 others associated with endocarditis, accumulation of cells with necrosis was found in the myocardium in such an arrangement as to suggest Aschoff nodules. The possibility that myocardial changes may lead to circulatory failure is hardly mentioned.

In the following, 2 cases of meningococcus myocarditis will be reported briefly. In 1 case the disease was fulminating and took a rapid course, the patient having been ill only about 50 hours. In the 2nd case the patient had apparently improved from a meningococcus meningitis, but later developed signs of heart failure and died.

### CASE REPORTS

**CASE 1.** An adult white male, 19 years old, was admitted in a comatose condition. The history revealed that he was in good health up to 1 day before admission, but gradually began to feel drowsy and lapsed into coma. He had had momentary dizzy spells for 2 to 3 weeks prior to admission.

Physical examination revealed small hemorrhagic spots all over the body and in the conjunctiva. The neck was rigid. The Kernig reaction was positive bilaterally. The heart was not enlarged but there was an inconstant diastolic blowing murmur at the apex. The temperature was 39° C. The pulse rate was 124

beats per minute and the arterial blood pressure 100/70. The white blood count was 26,100. There were 80 per cent neutrophilic polymorphonuclear leukocytes, 8 per cent lymphocytes, and 12 per cent immature leukocytes. A spinal puncture was performed and 15 cc. of a thick purulent liquid were removed. The cerebrospinal fluid contained 18,500 cells per cmm. Smears revealed Gram negative intracellular biscuit shaped diplococci which morphologically showed the characteristics of meningococci.

The content of glucose of three successive specimens of cerebrospinal fluid was 0, 10 and 18 mg. per 100 cc. respectively.

The patient became markedly cyanotic and died 30 hours after admission. The clinical diagnosis was meningococcus meningitis. The possibility of the presence of a meningococcus endocarditis was also considered.

### *Postmortem Examination*

Autopsy revealed an acute purulent leptomeningitis; cloudy swelling of the liver and kidneys; passive hyperemia of the liver; an early bronchopneumonia; and petechial hemorrhages of the skin, pericardium and pelvises of the kidneys. There was a generalized hyperplasia of the lymph nodes. The heart findings will be given in more detail.

The heart was slightly enlarged, weighing 375 gm. The pericardial cavity contained 150 cc. of seropurulent liquid and the surfaces were dull, finely granular and contained a small amount of fibrin. The mural endocardium was smooth and glistening and there were no thrombi. A few petechial hemorrhages were found throughout the endocardium but were particularly pronounced in the region of the interventricular septum close to the base of the aortic valve. The myocardium on section was grayish red and showed a number of red dots and reddish gray streaks.

### *Histological Examination*

Histological examination of the heart showed an epicardium that was covered with fibrin and enmeshed polymorphonuclear leukocytes. The subepicardial vessels were markedly dilated. The subendocardial portions, particularly in the region of the bundle of His, showed many extravasated red blood corpuscles separating the individual fibers from one another. Scattered throughout the myocardium were a number of polymorphonuclear leukocytes, mainly arranged in groups, which interrupted the course of the heart muscle fibers and also extended into the interstitial tissue. A number of red blood cells and large endothelial leukocytes were also

found in these regions which showed the very recent inflammatory exudate. Occasionally large cells with basophilic granules were observed in the Giemsa preparations. Throughout the sections necrotic heart muscle fibers were recognized, which were separated from one another by polymorphonuclear leukocytes, red blood cells and endothelial leukocytes. Some of the perivascular spaces contained deposits of fibrin and a few polymorphonuclear leukocytes. Outspoken infarcts were not observed, but there were minute and larger areas of necrosis which were infiltrated by polymorphonuclear leukocytes. The Gram-Weigert stain revealed a number of biscuit shaped diplococci. They were, in part, intracellular within the polymorphonuclear leukocytes, and in part extracellular. The Rudnikoff-Stawsky<sup>23</sup> modification of the Gram stain indicated that they were Gram-negative. The muscle fibers throughout showed indistinct striations. The intercalated discs were prominent.

### *Summary*

A 19 year old male who developed a meningococcus meningitis died abruptly after developing a severe cyanosis. The autopsy revealed, in addition to the meningococcus meningitis, an acute myocarditis which undoubtedly was the result of a meningococcus septicemia.

CASE 2.\* A 22 year old female was admitted to the hospital complaining of drowsiness, headache, vomiting, fever of 2 days duration and a body rash. She was perfectly well until 3 days before admission.

Physical examination revealed an acutely ill, white female. The temperature was 38.5 C. The pulse beats were 132 per minute with extrasystoles; the arterial blood pressure was 114/85. The neck was rigid and any motion of the head caused intense pain. There was impaired resonance over the right lung. The heart was within normal limits and there was a systolic murmur over the pulmonary area and the upper sternum. The electrocardiogram revealed a sinus tachycardia. Examination of the blood showed 3,920,000 red blood cells. The hemoglobin was 70 per cent. There were 25,900 leukocytes, 95 per cent of which were neutrophilic polymorphonuclear leukocytes, and 5 per cent lymphocytes. The urine contained traces of albumin. A spinal puncture was performed and 30 cc. of cloudy fluid removed. The content of glucose of the cerebrospinal fluid varied from 13 to 46 mg. per 100 cc. The fluid contained 18,000 polymorphonuclear leukocytes, and smears revealed intracellular Gram-negative diplococci which showed the morphological characteristics of meningococci. Meningococci were cultured from the cerebrospinal fluid. The organisms were also identified

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\* This case is referred to in the chapter on meningitis by Levinson in Practice of Pediatrics by J. Brennemann. W. F. Prior Co., Inc., Hagerstown, Md.

by agglutination with a standard antimeningococcus agglutinating serum on two occasions in dilutions of 1:160 and 1:320 respectively. The blood culture taken on one occasion remained sterile.

The patient was given intraspinal and intravenous injections of antimeningococcus serum. After a temporary improvement, when she was thought to have recovered from the meningitis, she developed subconjunctival ecchymoses, gradually became cyanotic and died 30 days after admission.

### *Postmortem Examination*

Autopsy revealed an acute fibrinopurulent meningitis; acute meningo-encephalitis; thrombosis of the meningeal veins and longitudinal sinus; cloudy swelling of the liver and kidneys; acute splenic hyperplasia; a recent bronchopneumonia; and an interstitial pneumonia. There was also passive hyperemia of the lungs, liver, spleen and kidneys.

The heart was about normal in size and shape, weighing 300 gm. A few mural thrombi were found in the right auricle. The valvular apparatus was intact. The subendocardium of the left ventricle in the region of the interventricular septum showed several petechial hemorrhages. The myocardium throughout was grayish and of a "boiled" appearance.

### *Histological Examination*

Histological examination of the myocardium showed swollen heart muscle fibers with indistinct striations. The subendocardial regions corresponding to the interventricular septum of the left ventricle showed many extravasated red blood corpuscles which were found more or less confined to the bundle of His. Close to the hemorrhagic zones a number of polymorphonuclear leukocytes were present infiltrating the interstitial tissue between the muscle fibers. Only débris of the latter was found in these regions. In other fields the polymorphonuclear leukocytic infiltration was more diffuse, involving both the parenchyma and the interstitial tissue. A number of endothelial leukocytes and many red blood corpuscles were found among the polymorphonuclear leukocytes. There were also present large cells with coarse basophilic granules and rarely a few lymphocytes. In a few regions the inflammatory cells were confined to the perivascular spaces. The Gram-Weigert stain revealed a number of biscuit shaped diplococci, partly intra- and partly extracellular. The Rudnikoff-Stawsky<sup>23</sup> modification of the Gram

stain showed that the organisms were Gram-negative. A culture from the myocardium was not taken. Neither the endocardium nor the pericardium revealed histological changes.

### Summary

A 22 year old female was admitted with symptoms of meningitis. She seemed to improve from the meningitis but gradually developed cyanosis and died. At autopsy, in addition to a meningo-encephalitis, an acute myocarditis was found which was the result of the meningococcus infection.

### COMMENT

These 2 cases are characteristic, inasmuch as they clinically revealed evidence of circulatory failure and anatomically a myocarditis. In the 1st case the circulatory collapse occurred rapidly, the patient having been ill only 2 days, but in the 2nd the circulatory failure developed rather slowly. Both patients showed meningococcus meningitis. In the 1st case the culture of the cerebrospinal fluid remained negative. The short stay of the patient in the hospital prevented obtaining another specimen. Smears from the cerebrospinal fluid, however, showed the characteristic Gram-negative intracellular biscuit shaped diplococci. Because of this finding and the presence of the intracellular Gram-negative diplococci in the inflammatory lesions of the myocardium, it seems clear that the cause of the myocarditis was a meningococcus bacteremia, even though absolute proof is missing. In the 2nd case a pure culture of meningococcus was obtained from the cerebrospinal fluid, in addition to finding the organism in the smears from the cerebrospinal fluid and in the sections of the heart muscle.

As stated above, observations on meningococcus myocarditis are exceedingly rare. Relatively more common a type of myocarditis is reported, which occasionally is found associated with meningococcus endocarditis, though this type of endocarditis *per se* is also a rare occurrence. In none of the reported cases, or in these 2 instances, was the myocarditis diagnosed grossly or was it even suspected.

Histologically the myocarditis is characterized by the hemorrhagic exudate, the early appearance of large endothelial leukocytes, the early destruction of muscle fibers and the presence of intracellular Gram-negative diplococci. The foci of necrosis are similar to those

seen in the myocardium in instances of subacute bacterial endocarditis.<sup>24</sup> Outspoken abscesses were missing. In neither of the two hearts were granulomatous lesions seen similar to those described by other authors. Although in both hearts the lesions were more or less diffuse, in each the bundle of His was involved, revealing polymorphonuclear leukocytic infiltrations and hemorrhage. Attempts toward healing were not observed.

Since the 1st case of meningococcus myocarditis reported was observed, special attention has been given to the myocardium of 9 additional cases of meningococcus meningitis. One of these constitutes the 2nd case here reported. In other words, meningococcus myocarditis was found twice among 10 cases of meningococcus meningitis. This fact is in contradiction to the prevailing opinion as to the extreme rarity of meningococcus myocarditis which one is forced to accept after study of the pertinent literature. Meningococcus myocarditis perhaps would be considered less rare if the myocardium of relevant cases was examined more carefully.

In view of the prevailing opinion that the primary lesion in meningococcus meningitis is a meningococcus bacteremia (Herrick<sup>11</sup>), it is not surprising that occasionally meningococci may be the cause of an isolated myocarditis. It is likely that the associated myocarditis with resulting myocardial failure seriously influences the prognosis, not only because of the impending myocardial failure but also because it seems that the myocarditis is the result of an overwhelming infection with meningococci. The abrupt death of the first patient may be explained by the overwhelming infection with meningococci, resulting in myocarditis.

#### SUMMARY AND CONCLUSIONS

Observations on isolated meningococcus myocarditis not accompanied by meningococcus endocarditis are rare, as far as one can judge from the relevant literature. However, because of the fact that *meningococcus myocarditis* was found twice among 10 cases of meningococcus meningitis, which were carefully studied for inflammatory changes in the heart muscle, the disease is apparently not so rare as one would expect from the literature.

Two cases of meningococcus myocarditis are reported. In 1, the patient developed signs of meningitis and died with marked cyano-

sis about 50 hours after the onset of symptoms. The 2nd patient, who also had typical symptoms of meningitis, had apparently improved when cyanosis developed and death occurred unexpectedly.

Histologically meningococcus myocarditis is characterized by a hemorrhagic exudate, the early appearance of endothelial leukocytes, destruction of muscle fibers and the presence of intracellular Gram-negative diplococci. There are also foci of necrosis which, however, are similar to those seen in the myocardium of cases of subacute bacterial endocarditis.

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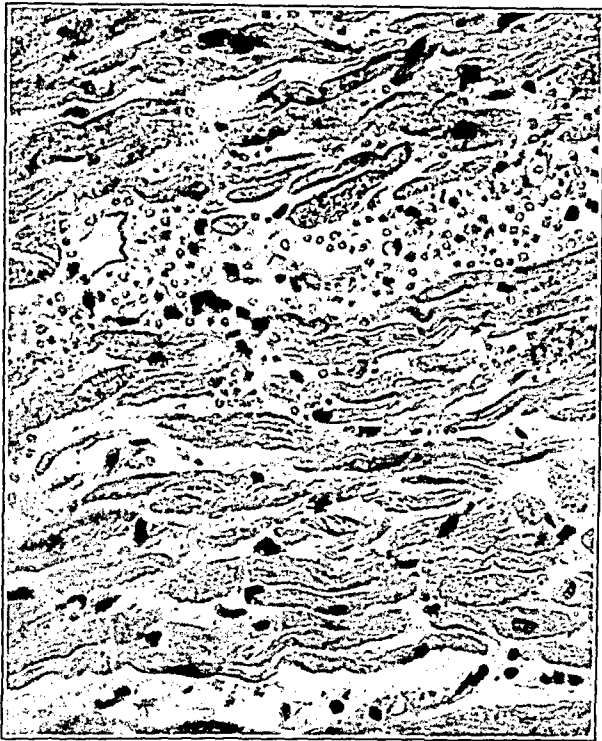
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## DESCRIPTION OF PLATE

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### PLATE 123

- FIG. 1. Note the necrotic heart muscle fibers and the hemorrhagic exudate. Iron hematoxylin-eosin preparation.  $\times 350$ .
- FIG. 2. Note the fibrin in the perivascular space. Iron hematoxylin-eosin preparation.  $\times 300$ .
- FIG. 3. Acute myocarditis. Note destruction of heart muscle fibers. Iron hematoxylin-eosin preparation.  $\times 800$ .
- FIG. 4. Note the intracellular cocci. Gram-Weigert stain.  $\times 1800$ .



1



2



3



4



# THE RELATION OF DIET TO THE OCCURRENCE OF GASTRIC LESIONS IN THE RAT \*

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Hyperplastic and ulcerative lesions have been observed in the stomachs of rats which we feel should be thoroughly investigated as to etiology, not only because an explanation of their cause might contribute to the knowledge of proliferative lesions in general, but also because an understanding of the mechanism of their development might disclose distortions in alimentation which accompany the formation of proliferative and ulcerative lesions in the stomach. A brief résumé of previous investigations concerning these lesions will indicate the confusion that exists regarding their etiology. They were first described in 1913 by a British investigator, Singer,<sup>1</sup> who believed them infectious in origin. He reported their presence in the stomachs of rats because Fibiger<sup>2</sup> was at that time working on the relation of Spiroptera to gastric carcinoma in the rat and similar lesions were described as precarcinomatous. It was not until 1923, however, that Pappenheimer and Larimore<sup>3</sup> demonstrated for the first time that the lesions were in some way related to a deficiency in the diet. They did not discover the exact nature of the deficiency but they did find that neither cod liver oil, nor Osborne and Wake-man's yeast extract, nor a change in the mineral content protected against these lesions.<sup>4</sup> Pappenheimer and Larimore felt, however, that the ingestion of hair was a contributory factor. On the other hand, Fujimaki, Kimura, Wada and Shimada<sup>5</sup> (1927) believed that the lesions were caused by a deficiency of vitamin A, and in this conclusion Santi<sup>6</sup> and Bisceglie<sup>7</sup> have agreed. If, however, a deficiency of vitamin A were the etiological factor one would expect that Wolbach and Howe,<sup>†</sup><sup>8</sup> who carefully studied the pathological

\* Received for publication February 20, 1936.

† In the experiments of Wolbach and Howe the animals were forced to eat all the rations each day in spite of loss of appetite from the vitamin deficiency. The animals did not suffer from other food deprivations therefore. Such precautions were not reported by the other investigators studying vitamin A deficiencies.

changes accompanying A avitaminosis, would have noted them, but instead they found only atrophy of the mucosa of the stomach.

A new suggestion as to the etiology of the lesions was recently proposed by Hoelzel and DaCosta,<sup>9</sup> and Carlson.<sup>10</sup> They suggested that the low protein content of the diet was responsible, for the lesions did not develop when large amounts of casein were given. The adequacy of vitamin control in their experiments, however, was not mentioned. Alternate days of fasting, they found, increased the number and severity of the lesions, and the lesions occurred in the glandular portion of the stomach as well when the deficiency was well advanced.<sup>10</sup> Steel filings incorporated in the diet as an irritant in place of hair did not produce the lesions when the diet was adequate, but when it was inadequate irritation made the lesions more extensive.

Büchner, Siebert and Molloy<sup>11</sup> produced the lesions in rats by increasing their gastric secretion with injected histamine. The character of diet given to these animals was not mentioned, but it was said that alternate days of fasting increased the incidence of the lesions, that hair in the diet did not produce them, and that when the rats ate feces no lesions resulted in spite of the injections of histamine. Lastly, Findlay<sup>12</sup> has described papillomatous proliferation in the squamous epithelium of the rumen portion of the stomach with B<sub>2</sub> deficiency but not with a B<sub>1</sub> deficiency.

From this brief review of the literature it should be apparent that the exact nature of the deficiency of the diet which produces the lesions has not been solved and that the problem is worthy of further investigation because of the possible relation of diet to tissue hyperplasia.

#### EXPERIMENTAL

The general plan of our investigations was to produce the lesions with a deficient diet in a definite length of time and then to add to it various factors in order to discover which ones would provide protection. The influence of the age of the animal on the production of the lesions in relation to the diet was controlled by studying the occurrence of the lesions in both young and adult rats fed on the different diets. The young rats used were 6 weeks old, while the adults were over 4 months of age. Both albino and hooded rats were employed in order to eliminate the possibility of a strain of

rats particularly susceptible to the development of the lesions. They were kept in separate wire-bottom cages and 10 were fed each diet. The entire data for the experiments, including the weights of the animals taken at the beginning of the experiment and when killed, are given in Table I. At autopsy the stomachs were removed, distended with formalin and allowed to fix before they were examined. This technic facilitated the study of the normal microscopic anatomy of the stomachs and the pathological changes therein.

For controls, 75 rats were fed an adequate stock diet.\* The stomachs of all these animals were without lesions. Microscopic sections were made of the various portions of these stomachs in order to furnish a picture of the normal microscopic anatomy.

The lesions were then produced by feeding rats a modified Pappenheimer and Larimore diet consisting of flour and salt mixture (Diet 84).<sup>4</sup> After 42 days on this regimen 9 out of 10 young rats developed lesions in the stomach. At the beginning of the experiment they ate about 6 to 8 gm. of food daily, but as they continued on the diet their appetites fell off gradually until finally they consumed only about 4 to 5 gm. daily. Most of them developed xerophthalmia and failed to gain weight (Table I). Only two of the stomachs examined contained hair. Most of the lesions were in the rumen, but five were in the glandular portion of the stomach as well (Figs. 1 and 2).

While in this length of time Diet 84 readily produced the lesions in the stomachs of young rats, it did not do so in the stomachs of the adult rats. In fact, 10 adult rats fed Diet 84 daily for 61 days had no ulcers, but when the adult rats were starved every other day and fed Diet 84 on the alternate days, then 7 out of 10 developed lesions in the stomach in 49 days. Likewise, when the food intake of the adult rats was restricted to 5 gm. daily, 6 out of 10 developed lesions in 42 days. However, when the complete diet was fed to control rats in such a manner that the animals lost a corresponding amount of weight over the same length of time, then the lesions did not develop. It is obvious from these experiments that young rats are more sensitive to the deficiency of the diet than are adult rats and that weight loss alone does not account for the formation of the lesions.

\* Calf meal diet.

TABLE I

Experiment No.	No. of rats	How fed	Days on diet	Age	Diet	Weight changes	Lesions
Controls	50	Daily	50	Adult	Normal Zucker	+8%	0
"	25	Daily	Life span	Young	calf meal	..	0
<i>Lesion-Producing Diet — Diet 84</i>							
31	10	Daily	42	Young	84	-5%	9
37	10	Daily	61	Adult	84	-8%	0
11	10	Alt. days	49	Adult	84	-41%	7
Y1	10	Daily. Re- stricted 5 gm.	42	Adult	84	-35%	6
<i>Diets with Vitamins A-D</i>							
2	10	Daily	42	Young	84 + 5% CLO*	+14 gm.	5
20	10	Alt. days	50	Adult	84 + butter	..	4
21	10	Alt. days	50	Adult	84 + carotin	..	5
Y4	10	Alt. days	42	Adult	84 + 5% CLO	-22%	6
24	10	Daily	52	Young	Moise + CLO	+47.6 gm.	1
<i>Diets with Yeast</i>							
3	10	Daily	42	Young	84 + 5% yeast	+1%	0
Y8	10	Alt. days	42	Adult	84 + 5% yeast	-16%	1
Y9	10	Daily	42	Young	84 + 5% yeast	+72%	0
22	10	Alt. days	50	Adult	84 + 5% yeast	-12.2 gm.	3
29	10	Alt. days	52	Adult	84 + 10% yeast	-31 gm.	0
4	10	Daily	50	Young	84 + 5% CLO + 5% yeast	+2.3 gm.	0
18	15	Daily	44	Young	84 + 5% yeast + hair	..	1
Y2	9	Alt. days — restricted	42	Adult	84 + 5% yeast	-13%	4
<i>Miscellaneous</i>							
5	10	Daily	42	Young	84 + 20% casein	+11.3 gm.	3
6	8	Alt. starva- tion	40	Adult	84 + yeast + 20% casein + histamine	+2.6 gm.	0
Y113	10	Alt. starva- tion	42	Adult	84 + histamine	-28%	5
Y16	10	Alt. starva- tion	42	Adult	84 + feces	..	0

\* CLO = cod liver oil.



*Pathology of the Lesions*

In the rumen portion of the stomach the smaller lesions were, grossly, round ulcerated areas with heaped up, light yellow circumferences. The edges were not undermined. The more extensive ones were more irregular in shape and tended to have one longer axis. The blood vessels, most prominent on the serosal surface, were more numerous around the lesions than elsewhere and radiated in a stellate pattern from the defects. Food particles frequently adhered to the ulcerated surfaces. Between the ulcers yellowish plaques of tissue of firmer consistence were found and these were sometimes so numerous that they thickened the entire rumen portion of the stomach. The ridge between the squamous and glandular portions of the stomach was frequently hypertrophied (Figs. 1 and 2).

Microscopically the ulcerations occurred in hypertrophied squamous epithelium; that at the margins and that of the plaques was hyperplastic, thickened and often reduplicated on itself. There was exfoliation of the keratin layer (Figs. 3 and 4). The processes of squamous epithelium extending downward toward the muscularis mucosa were widened and frequently caused thinning of this structure. Instances have been reported where the continuity of the muscularis mucosa has been broken by these downward extensions.<sup>5</sup> The subjacent connective tissue both above and below the muscularis mucosa was edematous and in some areas infiltrated with leukocytes, especially near the ulcerations. Mitotic figures in the epithelium were numerous, but the cells were not anaplastic. Superficial necrosis, not extensive enough to cause ulceration, had also occurred on the surface of some of the hyperplastic plaques. The surfaces of the ulcers were, microscopically, covered with the débris of keratin, dead epithelial cells and leukocytic fragments. There was no regenerating fibrous tissue seen in the bases of the ulcers nor was there regeneration of epithelium at the edges of the defects. The ulcerations had, therefore, occurred recently.

In the glandular portion of the stomach the incidence of the lesions was greatest in the pyloric antrum, but they were found over the entire secreting area (Fig. 2). In general, they were smaller and more punched out than those in the rumen, but their edges were also heaped up. Microscopically the individual glands of the epithe-

limum were elongated, their ends blunted and separated. The ulcerations occurred in hyperplastic areas and consisted of craters with steep walls (Fig. 5). Cellular infiltration adjacent to the ulcers was common. The non-hyperplastic areas of epithelium showed no evidence of gastritis similar to that seen in the gastric mucosa of the human stomach with peptic ulcer.

It is apparent, therefore, that the pathological changes were the same in both the rumen and the glandular portions of the stomach, consisting essentially of areas of hyperplasia with ulceration.

### *Relation of Vitamins A and D to the Development of the Lesions*

To Diet 84 adequate amounts of vitamins A and D were supplied by the addition of 5 per cent cod liver oil. The young rats fed on this mixture for 42 days ate and grew better than those on Diet 84 alone, but 5 out of 10 of them developed lesions in the stomach. Likewise, 6 out of 10 adult rats starved every other day and forced to take cod liver oil on the alternate days developed the lesions. Carotin and butter, employed as sources of vitamin A, also failed to provide protection against the lesions even though the rats were forced to eat these substances separately. It was therefore concluded that vitamin A did not protect against the lesions.

### *Effect of Whole Yeast*

Five per cent whole yeast was then added to Diet 84 to supply the B complex. The 10 young rats fed on this mixture were without lesions in the stomach, but 1 of the adult rats starved every other day and fed Diet 84 plus 5 per cent whole yeast on the alternate days had a lesion. The experiment was, therefore, repeated with 10 other adult rats, and 3 developed lesions. For a second trial, young rats were given a different brand of whole yeast (5 per cent), but again no lesions developed. Next, adult rats, starved every other day, were given 10 per cent whole yeast and then absolute protection followed. Moreover, 5 per cent whole yeast plus 5 per cent cod liver oil added to Diet 84 gave absolute protection, and even when the bulk of Diet 84 was increased 25 per cent by the addition of hair, only one lesion developed when 5 per cent whole yeast was given to young rats.

Lastly, when adult rats were fed a sufficient amount of whole

yeast, lesions did not develop although histamine had been injected according to the technic of Büchner. The Osborne and Wakeman yeast extract added to Diet 84, however, did not give protection as Pappenheimer and Larimore had likewise found (Table I). We were, therefore, forced to conclude that whole yeast when given in sufficient amounts can prevent the development of these gastric lesions.

That this protection by whole yeast is not absolute, however, was not only brought out by the above experiments but also by the fact that when the amount of flour and salt mixture was restricted, 5 per cent whole yeast afforded less protection than when a liberal amount of Diet 84 was allowed. Thus, with a restricted amount of Diet 84 plus 5 per cent whole yeast, 4 out of 9 adult rats developed lesions while only 3 of them had lesions when a liberal amount of Diet 84 was allowed. And again, when the diet was made more complete with the addition of other food factors, then the lesions were less frequent although the amount of yeast was kept constant. Thus only 1 young rat developed a lesion after 52 days when cod liver oil and Crisco were added to Diet 84 plus 5 per cent yeast. These facts indicate that the more complete diet has a sparing action on the necessary food element.

#### *Effect of Increased Amount of Protein in the Diet*

Finally, because of the work of Hoelzel and DaCosta,<sup>9</sup> 20 per cent vitamin-free casein was added to Diet 84 and fed to young rats daily for 42 days but, when killed, 3 of these animals had lesions in the stomach. On the other hand, with 5 per cent whole yeast added to this mixture no lesions developed.

#### DISCUSSION

The experiments indicate that whole yeast added to a deficient diet protects against the development of hyperplastic and ulcerative lesions in the stomach of the rat; that young rats are more sensitive to the deficiency of the diet than adults; and that other food factors contributing to the adequacy of the diet have a sparing effect. It follows, accordingly, that when the diet is adequate a longer time is required to produce the lesions with the necessary substance absent than when the diet is less adequate. The amount of neces-

sary substance required to give absolute protection under any circumstance will, in the last analysis, therefore, depend on the age of the animal and the severity of the depletion produced by the experiment and this, in turn, on the manner of feeding and the general adequacy of the food given. The dietary regimen preceding the initiation of the depletion experiment is, of course, a determining factor.

The exact nature of the protective substance in yeast must further be investigated, but the action of one of the contained vitamins is immediately suggested. Carlson<sup>10</sup> has advanced the theory that the lesions were caused by gastric stasis, but such a fact, if true, would only support the contention that the lesions were produced by a vitamin deficiency. The withdrawal of vitamin B from a diet decreases gastric motility while the addition of an adequate amount of the vitamin to a deficient diet causes the motility to return to normal.<sup>13</sup> The greater sensitivity of young rats to an inadequate supply of the necessary substance is, of course, compatible with the picture of a vitamin deficiency, and there is the possibility that other foods might contain traces of the necessary vitamin. Thus, if casein were not specially treated so as to destroy the vitamin content then it is conceivable that the addition of large amounts to a diet would afford protection. This might be the explanation of the discrepancy between this work and that done by Hoelzel and Da Costa.<sup>9</sup> On the other hand, the sparing action of cod liver oil is difficult to explain on a vitamin basis, especially when the oil itself, as shown by Pappenheimer and Larimore, has the same effect after the vitamin content is destroyed.

Actually the discrepancies between our experimental results and those obtained by the previous investigators are not difficult to explain nor are they as great as would be imagined. Some differences have naturally arisen from the failure to control the influence of the age factor and further from the assumption that the mere presence of a food in a diet necessarily guarantees that the desired nutritional benefit will be obtained. For benefit or protection, as the case happens to be here, the necessary substance must be consumed and in adequate amounts, as our investigations have shown. Thus, with the loss of appetite which accompanies prolonged A avitaminosis, there would be an insufficient ingestion of the necessary substance even though yeast were present in the diet, and the lesions

should develop.<sup>5</sup> The Osborne and Wakeman yeast extract previously used by Pappenheimer and Larimore did not contain the necessary factor,<sup>4</sup> so it is quite understandable why these investigators did not suspect the presence of a necessary substance in whole yeast. Findlay, though, in confirmation has described the formation of proliferative lesions in the stomachs of rats fed on a vitamin B-free diet and further demonstrated that the giving of B<sub>1</sub> did not prevent their development.

Pappenheimer and Larimore, and Carlson, have demonstrated that the incidence of the lesions is heightened by the presence of local irritation, and histamine with its resulting secretion of free acid into the empty stomach does irritate the gastric mucosa as Büchner has shown.<sup>11</sup> In Büchner's experiments, therefore, there is the possibility that the repeated flow of juice simply precipitated the lesions in animals previously inadequately fed, or, at least, we were unable to produce the lesions with histamine injections after the animals received sufficient quantities of yeast. Moreover, the sporadic occurrence of the lesions in laboratory rats illustrates the fact that animals can unknowingly be fed in such a way that they are on the borderline of or actually in the deficiency which produces the lesions. Henning and Norpoth,<sup>14</sup> for example, found so many of the lesions in their laboratory rats that they believed Büchner was describing a common disease in the stomach of the rat. Yet, on the other hand, so many well fed animals have been examined particularly for the lesions by Carlson, ourselves and others (Fibiger examined over 2000 wild rats) that one must conclude that Henning's rats were likewise inadequately fed. His finding of the lesions in what he considered normal rats, however, demonstrates how easy it is to assume that an irritative agent produces the lesions when actually the previous diet of the animal is inadequate. Even Büchner's own data support the thesis that his animals were near the deficiency, for starvation aided the production of the lesions, and some of the rats starved and receiving no histamine likewise developed the lesions. Lastly, Büchner believed that feces protected against the lesions because they neutralized the gastric juice secreted after the histamine was injected, but we have obtained the same protection by adding 10 per cent dried feces to Diet 84. Under these circumstances the lesions were not produced by the inadequate diet

because of the presence of feces and yet there were no obvious distortions in the secretion of gastric juice.\* The reports, therefore, that feces may contain vitamin B suggest that the action of a necessary food element may again be the answer.

Dalldorf and Kellog,<sup>15</sup> and Büchner, have argued that peptic ulcer in man arises from conditions similar to those experimentally needed to produce the lesions in the stomach of the rat, while others have advanced the hypothesis that the mechanisms involved are similar to those necessary to produce the gastritis which accompanies peptic ulcer. The latter might be a possibility, but certainly there is no similarity between these lesions and peptic ulcer in man. These lesions are acute, are multiple, and the ulcerations occur not only in areas where the epithelium is hyperplastic but in squamous epithelium as well. It is of interest, however, that patients with pellagra have somewhat similar lesions in the squamous epithelium of the lower end of the esophagus and that experimentally hyperplasia can be produced by a dietary deficiency.

#### SUMMARY AND CONCLUSIONS

1. Hyperplasia, hyperkeratinization and ulceration of the squamous epithelium of the rumen of the rat's stomach result from an inadequacy in the diet.
2. Similar lesions without keratinization occur in the glandular epithelium as well. The importance of these lesions lies in the relation of diet insufficiencies to tissue hyperplasia.
3. Young rats are more sensitive to the inadequacy of the diet than are adult rats. Adult rats on the same diet must be starved every other day in order to develop the lesions in the same length of time.
4. An adequate intake of whole yeast prevents the formation of the lesions in both young and adult rats. Additional amounts of vitamin-free casein, or cod liver oil, or other forms of vitamin A do not prevent the formation of the lesions. Ingestion of feces prevents their occurrence.
5. The relation of these lesions to peptic ulcer and to previous investigations of their etiology is discussed.

\* Bürkle-de la Camp (*Deutsche Ztschr. f. Chir.*, 1929, 220, 31) has repeated Büchner's investigation and obtained the same result, so the rôle of the gastric juice must be considered important in spite of the above criticism.

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## DESCRIPTION OF PLATES

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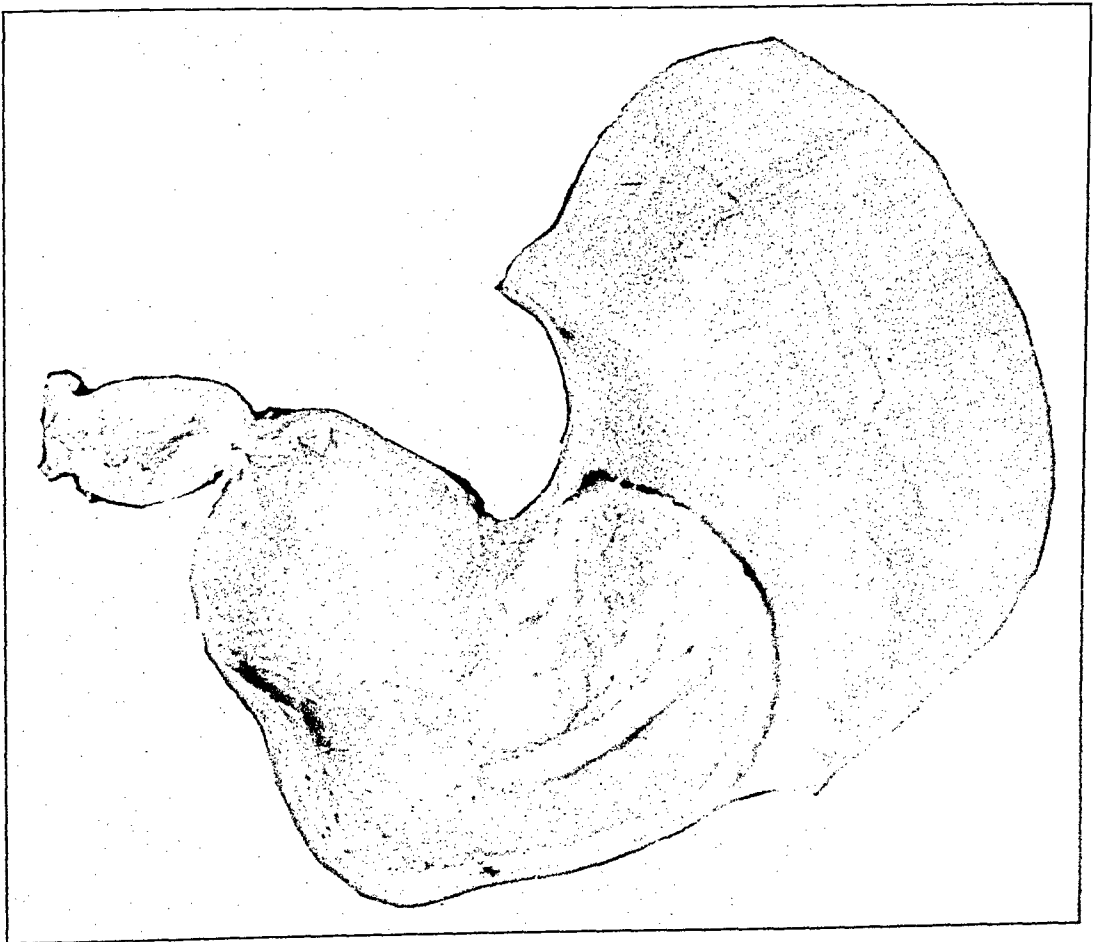
### PLATE 124

- FIG. 1. Extensive lesions of the rumen. Almost the entire area is hyperplastic, but several ulcerations can be seen.
- FIG. 2. Lesions in the glandular portion of the stomach. Note that the entire rumen has the normal smooth appearance (contrast with Fig. 1) except that there is a small lesion beginning in the lower right hand corner. The hypertrophy of the juncture between the two portions of the stomach is clearly shown.





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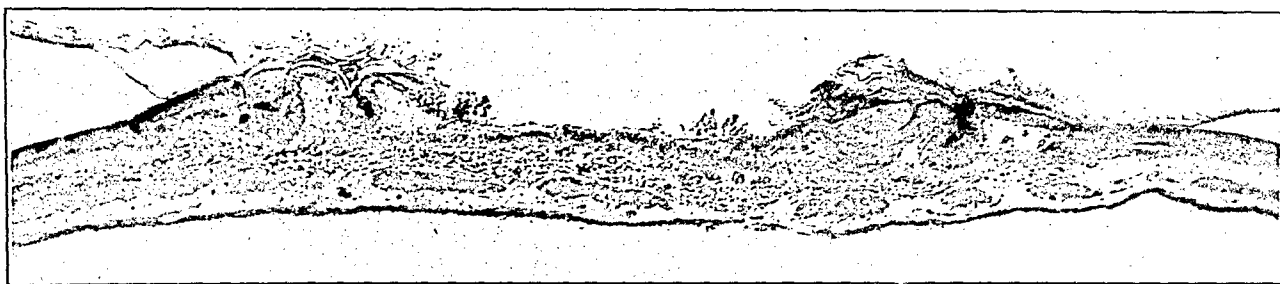
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PLATE 125

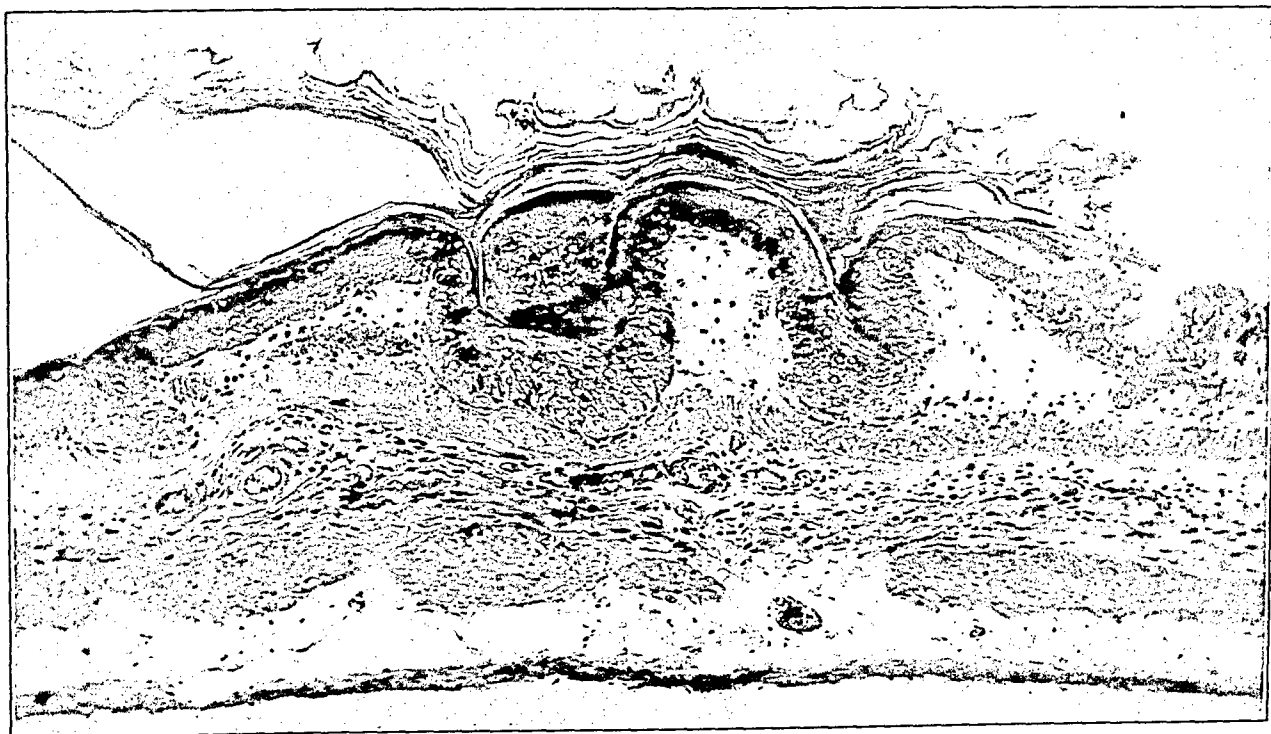
FIG. 3. Low power magnification of an ulcerated area of the rumen surrounded by hyperplastic squamous epithelium. The normal thickness of epithelium and the normal architecture of tissue layers can be seen at the edges of the section.

FIG. 4. High power magnification of the squamous epithelium at the edge of an ulceration in the rumen. Hyperkeratosis, the increase in thickness and the reduplication of the epithelium into blunt papillary processes pressing on the muscularis mucosa are evident. Edema of the connective tissue between the papillary processes is also apparent. Necrosis of the epithelium at the edge of the ulceration is seen on the right.

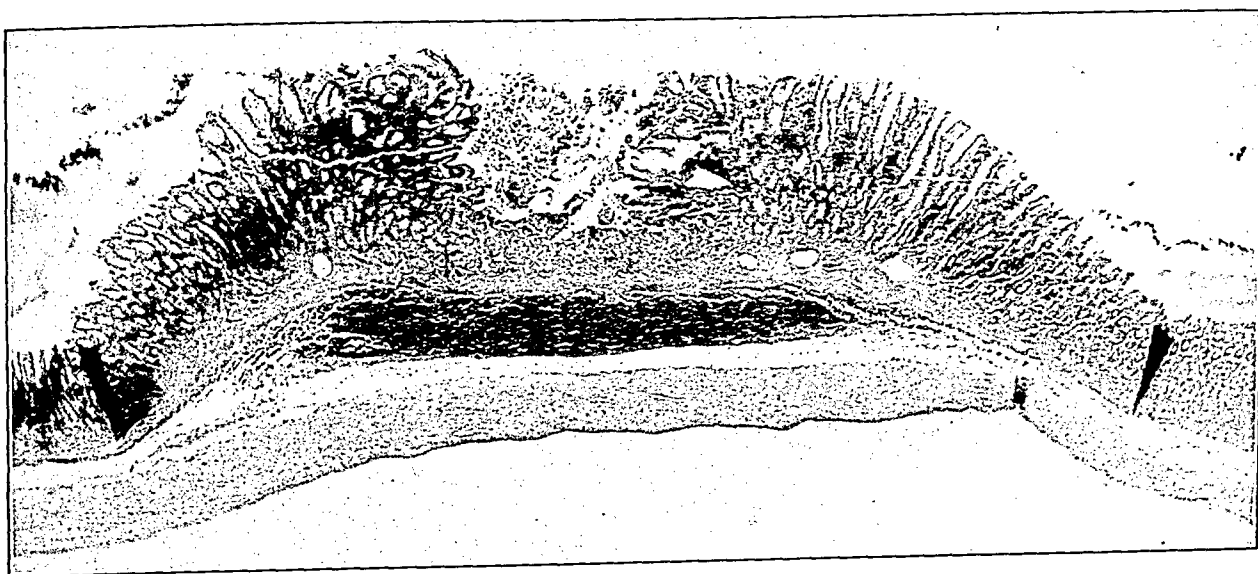
FIG. 5. Ulceration in the glandular portion. The ulceration has not extended to the muscularis mucosa and beneath it a collection of lymphocytes is seen. The normal architecture of the epithelium can be seen on either side of the ulceration.



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## AMYLOIDOSIS OF LUNGS AND HEART\*

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The subject of amyloidosis of the lungs has received relatively little attention in the medical literature. Virchow<sup>1</sup> in 1857 was apparently the first observer to mention the condition. He described generalized amyloidosis occurring in an aged female suffering from chronic Bright's disease. Small amounts of hyaline substance were present in the small blood vessels and the alveolar walls of the lungs. Iodine followed by sulphuric acid gave reactions typical of amyloid.

In 1909 Schilder<sup>2</sup> presented the findings from 2 cases of chronic pulmonary tuberculosis in which there were widespread amyloid deposits. There was moderate involvement of the blood vessels and alveolar walls of the lungs.

Tsunoda<sup>3</sup> in 1910 stated that of 7 cases of general amyloidosis investigated only 3 had slight amounts of amyloid in the pulmonary vessels and alveolar septa.

The first general discussion of amyloidosis of the lung was made by Kschischo<sup>4</sup> in 1912. He reviewed the cases reported up to that time and added a case of his own, in which there was a considerable amount of amyloid in the spleen and kidneys, and a slight amount in the liver. In the lungs deposits were found in the alveolar septa and in the medium sized vessels. A chronic inflammatory process believed to be syphilitic was present also in the lungs.

Glaus<sup>5</sup> in 1917 described a case of multiple myeloma. There was amyloidosis of the whole intestinal tract and deposits of the substance in the mesenteric vessels, heart, striated muscle, and myeloid tumor of the bone marrow. In places the pulmonary alveolar walls were thickened, in part because of circumscribed, and in part on account of diffuse masses of amyloid.

Lubarsch and Plenge<sup>6</sup> in 1931 stated that 80 cases of amyloidosis at the Berlin University Pathological Institute and 24 cases at the Hindenburg Hospital at Zehlendorf were studied with special care

\* This paper was read before the meeting of the New York Pathological Society, January 23, 1936.

Received for publication March 25, 1936.

to determine the frequency of amyloid involvement of the lungs. Of the total of 104 cases, only 9 presented any evidence of amyloid in the lungs, and in only 1 of these, a patient with chronic pulmonary tuberculosis, was it at all extensive.

In most of these cases there was a concomitant or preceding chronic disease as an etiological factor for the amyloid and the involvement of the lungs was usually part of a general amyloidosis. A brief description of a case which may be considered as a somewhat atypical example of this form of the disease follows. Localized amyloidosis of one lung and fairly extensive deposits of amyloid in the spleen were present. An extensive carcinoma of the bladder and a localized bronchiolectasis of the affected lung were possible etiological factors.

#### REPORT OF CASE

**CASE 1. Clinical History:** H. W., a white male, 78 years of age, entered the department of urology (James Buchanan Brady Foundation) of the New York Hospital Nov. 11, 1935. He had had many of the diseases of childhood but was otherwise well until about 4 years before admission to the hospital when a prostatectomy was performed. In June, 1935, he entered the hospital because of urgency, frequency, nocturia and dysuria of 1 years duration. A tumor 5 cm. in diameter, in the right side of the bladder, was removed by means of high frequency current. The tumor did not appear to invade the bladder wall. Radon seeds were placed in the base of the growth. In September, 1935, the suprapubic wound opened, but soon healed following bladder irrigations. Again, in November, 1935, there was a urinary fistula from which came a very foul urine. The bladder was irrigated continuously but the wound did not heal. The patient's blood urea nitrogen rose and 1 day before death there was no excretion of urine. He died Dec. 12, 1935.

#### *Postmortem Examination*

Autopsy (No. 8346) was performed 14 hours postmortem. The body was well developed and weighed 48 kg., although there was evidence of loss of weight. The appendix was bound down by fibrous adhesions, and other short fibrous bands extended between several adjacent loops of intestine. The left pleural cavity was obliterated by adhesions, particularly dense at the apex of the lung.

**Heart:** The heart weighed 370 gm. In the lower part of the left ventricle the wall was decreased in thickness and there was an increase of fibrous tissue. The remainder of the myocardium was not unusual in appearance. The wall of the anterior descending branch of the left coronary artery was thickened in one area and contained

calcium. In the lumen was a small thrombus. In the intima of other branches of the coronary arteries were small atheromatous plaques. The aorta contained a number of these plaques, some of which were calcified.

*Lungs:* The lungs together weighed 960 gm. The left lung was crepitant throughout and its cut surface was pink and relatively dry. The right lung was similar to the left in appearance, except for an area measuring approximately 5 by 3.5 by 2.5 cm. in the inferior and anterior part of the lower lobe. On section it was found to be somewhat wedge shaped, extending to the diaphragmatic margin, and was composed of firm grayish tissue, near the center of which were several irregular spaces. The largest of these measured about 1.5 cm. in greatest dimension, and several contained thick, yellowish white, purulent material. The cavities were lined by mucosa and communicated with dilated branches of the bronchi. At the apex of each lung was a fibrous scar of the pleura. In the left lung strands of fibrous tissue extended about 1.5 cm. below the scar into the pulmonary substance. A small calcified nodule was found in the lower lobe of the right lung just beneath the pleura of the interlobar fissure. There was a small calcified nodule in a deeply pigmented lymph node situated just above the right bronchus.

*Spleen:* The spleen weighed 130 gm. The surface was smooth and reddish purple. It was fairly firm and held its shape well on section. Small pale foci, apparently malpighian bodies, were fairly prominent in the light red or pink pulp. The trabeculae were not easily identified. When tincture of iodine was applied to the cut surface the pale foci became brown. These turned to greenish blue when concentrated sulphuric acid was added.

*Bladder:* The bladder wall was firm and in places measured 2.5 cm. in thickness. Most of the mucosa was replaced by soft, friable, opaque yellow tissue which invaded the wall. The ureteral orifices were not recognized. There were no metastases in adjacent lymph nodes.

### *Microscopic Examination*

*Heart:* There was a great increase of fibrous tissue in the lower part of the left ventricle and the walls of several small arteries were thickened by fibrous tissue. The lumen of one branch of the anterior descending branch of the left coronary artery was occluded by an

organized but recanalized thrombus, while in the other branch was a fresh thrombus.

*Lungs:* In general the alveoli were dilated. Many contained scattered polymorphonuclear leukocytes and a few large mononuclear cells. Leukocytes were also found in the walls of bronchioles. In the congested area of the right lower lobe masses of leukocytes were present in the lumens of dilated bronchioles and many lymphocytes and plasma cells were seen in the walls. Fibrous tissue was increased throughout this part of the lung. In addition there was a lighter staining hyaline material present in the walls of blood vessels and forming irregular masses or nodules in the alveolar walls. In areas the lumens of the alveoli were almost completely obliterated on account of the extensive deposits. Many multinucleated giant cells were seen at the margins of the amyloid masses, in places situated in indentations of the substance. In sections prepared by a combined Weigert's elastic tissue and Van Gieson's connective tissue stain the elastic fibers in the blood vessel and alveolar walls were distorted and in places had disappeared. The amyloid was stained dull yellow, in contrast to the bright red of the collagen. Gentian violet and iodine stains were unsatisfactory.

*Spleen:* Practically all of the lymphoid tissue of most of the malpighian corpuscles was replaced by a pink staining, homogeneous hyaline material, which was also present in places in the adjacent pulp. The walls of the arteries were thickened. Lymphocytes in the pulp were moderate in number. There were numerous plasma cells and scattered large mononuclear cells containing brown pigment granules. Gentian violet stain of a section of alcohol-fixed material gave a metachromatic stain for the amyloid and connective tissue.

*Bladder:* The innermost parts of the bladder wall were so necrotic that no cells were recognizable. More peripherally there were large masses of epithelial cells separated by delicate trabeculae of connective tissue. In other places the fibrous tissue was denser and more abundant and the cells formed small groups or strands. The epithelial cells varied in size, shape and staining properties with moderately hyperchromatic nuclei. A few multinucleated cells were seen. Mitoses were numerous. The tumor extended through the whole bladder wall.

The principal pathological changes in other organs were metas-



tases from the carcinoma of the bladder to the right adrenal; hydro-ureter, bilateral; hydronephrosis and chronic pyelonephritis, bilateral.

### *Comment*

Somewhat similar to this case are several reported instances of localized deposits of amyloid in the lungs. There was, however, no involvement of other organs. Herxheimer<sup>7</sup> in 1903 found large tumor-like deposits of amyloid in the lung of a male suffering from empyema.

Other examples of localized nodular amyloidosis of the lungs were described by von Werdt,<sup>8</sup> Meyer,<sup>9</sup> and Hallermann.<sup>10</sup> In von Werdt's case there was no obvious etiology. Meyer found generalized arteriosclerosis with atrophy of the kidneys, while in 1 of Hallermann's cases there was empyema and in the other pneumonia.

### ATYPICAL AMYLOIDOSIS

More frequently amyloid has been observed in the lungs in what has been called atypical or primary amyloidosis. This lesion is characterized, according to Lubarsch,<sup>11</sup> by the frequent absence of an associated disease ("Grundkrankheit"); the occasional nodularity of the deposits; the frequent failure of the deposits to react typically to the specific tests for amyloid; the almost complete absence of involvement of the usual organs; and, on the contrary, the unusual sites of deposit of the amyloid.

The following case of amyloidosis is atypical in a number of respects, and is especially unusual because of the remarkable quantity of amyloid in the lungs and heart.

### REPORT OF CASE

CASE 2. *Clinical History:* F. V., a white male, 52 years of age, was admitted to the medical service of the New York Hospital March 21, 1935. There was no history of significant disease in the family. A number of years before, he was treated for gonorrhea, complicated by urethral stricture. His general health since then had been good, however, until about 1 year before admission when he noted dyspnea on exertion and became more easily fatigued. These symptoms increased in severity during the next 9 months, and 3 months before admission he commenced to have night sweats and noticed some swelling and pulsation of the liver. There was also a chronic cough with production of a large amount of mucus. He was treated with digitalis and curtailed his activities,

but there was little improvement and 3 weeks before admission edema of the lower extremities appeared.

*Physical Examination:* The patient was well developed and nourished, but was moderately dyspneic. The percussion note was flat over the base of the right lung and breath sounds were absent in that location, as well as in the right axilla. The remainder of the lower lobe and the middle lobe were filled with moist râles. Similar râles were heard in the left lower lobe. The left border of the heart extended almost to the left anterior axillary line. The heart sounds were regular (70 per minute) and of tic-tac quality. A faint, soft systolic murmur was heard over the precordium. The systolic blood pressure was 125 and the diastolic 75 mm. Hg. The abdomen was slightly distended with the suggestion of a fluid wave. The liver edge was palpable a hand's breadth below the costal margin and was smooth and firm. There was slight swelling of the ankles.

*Course of Illness:* The urine contained 1+ to 2+ albumin and the specific gravity was 1.015 and 1.016 on two examinations. On one examination there were occasional white blood cells. The Kline exclusion and diagnostic tests were negative. The red and white blood cell counts were within normal limits. Four days after admission the blood urea nitrogen was 12 and the blood sugar 118 mg. X-rays of the chest showed an obscuring of both leaves of the diaphragm and the right costophrenic angle. The markings of the visible lung fields were accentuated and in places there was a punctate appearance. The heart shadow was definitely enlarged to the right and left, and the lower trachea displaced to the right.

The patient was given salyrgan intravenously and placed on a maintenance dose of digitalis. The dyspnea remained pronounced and from time to time a moderate amount of blood-tinged sputum was noted. Signs of consolidation of the lungs progressed and the pulse rate rose, varying between 71 and 120. The temperature ranged between 37° C. and 38.8° C., with general upward rise.

Death occurred 14 days after admission.

### *Postmortem Examination*

Autopsy (No. 8078) was performed 11 hours postmortem. The body was well developed and nourished and weighed 47 kg. Several teeth were carious. There were 300 cc. of clear yellow fluid with a specific gravity of 1.015 in the peritoneal cavity. In each pleural cavity were 600 cc. of slightly cloudy yellow fluid with a specific gravity of 1.010.

*Heart:* The heart weighed 650 gm. In both auricular appendages were masses of friable thrombus, lightly adherent to the endocardium. The valve measurements were normal. The myocardium was firm in consistence with a homogeneous, glassy cut surface. The right ventricle was 0.7 cm. and the left 2 to 2.5 cm. in thickness. There were a few atheromatous plaques in the intima of the coronary

arteries, and a few small lipoid deposits throughout the length of the aorta.

*Lungs:* The lungs weighed together 1710 gm. The pleural surface of each lung was roughened by minute tubercle-like elevations measuring in diameter from a fraction of a millimeter to about 4 mm. These small nodules were more conspicuous in the lower lobes, especially on the diaphragmatic surfaces where they gave the pleura a finely granular appearance. The lower lobes of both lungs were firmly consolidated and a dark bluish red. On section the cut surfaces of both lungs had a granular appearance on account of the presence of many pale, translucent, wax-like nodules. These were easily dislodged from the tissue by the tip of a knife and appeared then as small tapioca-like granules. They were particularly numerous near the diaphragmatic borders, but were also seen in the lighter colored, more crepitant upper lobes. A fibrous and calcified nodule about 1.5 cm. in greatest dimension was found near the apex of the right upper lobe beneath fibrous pleural adhesions. A main branch of the pulmonary artery supplying the left lower lobe was distended by a firm but friable, reddish gray blood clot. The tracheobronchial lymph nodes were much enlarged, dark red and friable, because of the presence of many small granules similar to those in the lungs.

*Spleen:* The spleen weighed 160 gm., was firm and a bluish gray. The cut surface was of somewhat glassy appearance and the malpighian corpuscles were indistinct.

*Gastro-Intestinal Tract:* In the mucosa of the stomach and duodenum were several small superficial erosions about 0.3 to 0.7 cm. in diameter, a few of which had hemorrhagic margins. In the mucosa of the jejunum were several small thickened areas, some of which had yellow opaque centers, the largest being about 0.4 cm. in diameter. There were hemorrhages in the wall beneath the lesions.

*Pancreas:* Not remarkable in appearance.

*Liver:* The liver weighed 1600 gm. The peritoneal surface was slightly granular with numerous small yellow foci. The organ cut with moderate resistance and the sectioned surface had mottled yellowish or pink lobular areas with central red foci. The gall-bladder was not unusual.

*Adrenals:* These were slightly larger than usual with a prominent translucent grayish medulla.

*Kidneys:* Each weighed 210 gm. and was firm and dark red. An excess of dark blood was scraped from the cut surface.

*Bladder, Prostate, Seminal Vesicles and Testes:* Not unusual.

*Organs of the Neck:* The thyroid and parathyroids appeared normal. A moderate amount of pinkish mucoid material was present in the trachea and larynx.

*Lymph Nodes:* The preaortic lymph nodes resembled the tracheobronchial lymph nodes already described. The mesenteric lymph nodes were somewhat enlarged, pale and friable.

*Brain and Spinal Cord:* Not examined.

*Iodine Test:* The iodine test for amyloid with Lugol's solution was employed on heart, lung, spleen and tracheobronchial lymph node. It was strongly positive on the heart and lungs, moderately positive on the spleen and only suggestive on the lymph node.

### *Microscopic Examination*

*Heart:* Practically every muscle fiber was surrounded by a zone of light pink staining material, in part homogeneous and in part fibrillar, causing a separation of the fibers from one another. The deposit was more abundant in the left ventricle. In each ventricle the muscle fibers had shrunk away from the surrounding substance. Some of the fibers were increased in size but many were greatly decreased in diameter. The small capillaries were surrounded by the pink matrix and this material was found in the walls of small arterioles, especially in the media and adventitia.

*Lungs:* A pink staining hyaline material was seen in the walls of blood vessels of varying size, both arteries and veins. The amount varied, in some instances being so extensive that little remained of the original structure of the vessels but a few strands of elastic tissue recognizable in a section stained by the combined Weigert and Van Gieson methods. In a number of vessels the internal elastic lamella could be seen to end abruptly in the deposits. In many instances it was not possible to distinguish arteries from veins, because of the degree of alteration. The larger vessels were less changed than the medium sized and small ones. The substance was also found in varying amounts in alveolar walls, in some places producing only a slight thickening. This minimum degree of involve-

ment was particularly noticeable in sections from the upper lobes. In other areas there were large irregular or rounded masses of amyloid, whose relation to the alveolar walls was difficult to determine, even with the aid of the elastic tissue stain. In some of the masses small fragments of elastic tissue were recognizable as remnants of the alveolar septa. Delicate strands of red staining collagenous tissue were also seen in the more homogeneous, dull orange colored amyloid. Lumens of one or more blood vessels were frequently found in the masses. The alveolar lumens in places were greatly reduced in size on account of compression by the hyaline material and in many instances were lined by cuboidal epithelium. A few alveoli were dilated. The lumens contained a number of large mononuclear cells with brown pigment granules in their cytoplasm. In places there was proliferation of fibroblasts, apparently in the larger septa. The blood vessels were congested; in the lower lobes the alveoli were packed with red blood cells and in a few places contained small amounts of fibrin and numerous polymorphonuclear leukocytes. Amyloid was present in the walls of small bronchi in the sections, especially in the mucosal layers. There were a few multinucleated giant cells at the edges of amyloid masses or in crevices within them. In some instances the cytoplasm of these cells was poorly differentiated from the amyloid.

*Spleen:* The malpighian corpuscles were replaced to a varying degree by a pink staining hyaline material. In addition this substance was found irregularly distributed throughout the pulp. Lymphoid tissue was greatly reduced in amount and red blood cells formed masses in between the amyloid deposits.

*Pancreas:* In the walls of small arteries there was a small amount of amyloid.

*Liver:* Extensive congestion and hemorrhage were present in the central parts of the lobules, with necrosis of some of the cells. There was a slight increase of connective tissue in the centers of the lobules. In many of the portal spaces the walls of the vessels, especially the portal veins, contained pink staining material.

*Adrenals:* In the inner part of the cortex, and to a slight extent in the medulla, there was a small amount of homogeneous, light pink staining material between the cells. A few of the vessels in and about the adrenal had similar deposits in the walls.

*Kidney:* A slight thickening of the walls of the capillaries of some of the glomeruli was seen and there was hyaline material in the walls of a few small arteries.

*Prostate:* The interstitial tissue was somewhat increased due in part to small masses of hyaline pink staining material, especially noted about small blood vessels.

*Intestine:* In localized areas in the mucosa, and especially the submucosa of the jejunum, were hyaline deposits in and around small blood vessels.

*Thyroid:* In a few areas a homogeneous material was present in the interstitial tissue and the walls of small vessels were frequently hyaline in appearance.

*Diaphragm:* Amyloid was present in the walls of a few of the smaller arteries but only a trace of the material was present between the muscle fibers.

*Lymph Nodes:* Section of a tracheobronchial lymph node presented almost complete replacement of the usual structure by homogeneous material. The follicles had disappeared and the lymphocytes were scattered here and there. Small blood sinuses were embedded in dense tissue. Mesenteric lymph nodes presented a similar picture.

*Cultures:* Postmortem cultures of the heart's blood, spleen and lung were sterile. Three rabbits, three guinea pigs and several white mice were injected subcutaneously and intraperitoneally with emulsion prepared from lung tissue. None presented any significant anatomical findings at autopsy several months later.

### *Comment*

In 1929 Lubarsch <sup>11</sup> described 3 cases in which the lungs were involved by amyloid. In 1 case there was extensive amyloid in the tongue, pharynx, esophagus and remainder of the gastro-intestinal tract, as well as in the heart and other organs, with the exception of the liver, spleen and kidneys. There were large amounts in the lungs, in many places in the form of solid nodules. In the 2nd case there was amyloidosis of the heart, tongue, esophagus and skin, with amyloid present only in the walls of arteries and veins of the lungs. In the 3rd case amyloid was abundant in the spleen, lymph nodes, prostate, seminal vesicles, epididymides, testes, stomach, intestines

and heart. There were "pinhead" to "lentil" sized nodules in the lungs. In the 1st and 3rd cases Lubarsch found no obvious underlying disease. In the 2nd case he believed that cystitis, pyelitis, furunculosis and recurrent valvular endocarditis could not be disregarded as possible factors. Physical signs suggestive of myocardial disease were present only in the 2nd case and were not of major significance.

Larsen <sup>12</sup> in 1930 cited the case of a man who had a typical history of failure of the left side of the heart, although death was due to a streptococcus septicemia. There were amyloid deposits in the heart and spleen and extensive diffuse involvement of the lungs. In the degree of involvement of the lungs and heart the 1st and 3rd cases of Lubarsch, and the case reported by Larsen, bear a fairly close resemblance to the 2nd case reported here.

There is another group of cases of atypical amyloidosis particularly with involvement of the heart and relatively insignificant involvement of the lungs. In 1886 Wild <sup>13</sup> presented a case of amyloidosis with especial involvement of the heart, but with some amyloid in the arteries and veins of the lungs and the mucosa and submucosa of the bronchi. There was no known predisposing disease. Clinically there was evidence of emphysema and weakness of the myocardium. Beneke and Bönning <sup>14</sup> in 1908 described the autopsy findings in an emaciated aged male who suffered from bronchitis. The clinical history was inadequate. Amyloid was present in the heart, and in small amounts in the lungs, but in no other organs. Koller <sup>15</sup> in 1932 presented a case of amyloidosis of the heart, tongue and bone marrow and small amounts of amyloid in the walls of the small blood vessels and capillaries of the lungs and in the alveolar septa. Diabetes was the only other disease present. Clinically there was cardiac insufficiency with edema of the lungs.

Three unusual cases of amyloidosis were reported by Bannick, Berkman and Beaver <sup>16</sup> in 1933. In only 1 of these cases were the lungs affected. There was generalized amyloidosis including involvement of the spleen, liver and kidneys. The walls of some pulmonary alveoli and of many blood vessels contained amyloid. The myocardium was somewhat friable and waxy, and microscopically showed a moderate amount of interstitial amyloid with atrophy of some of the muscle fibers. There were no cardiac symptoms. The etiology of the deposits was obscure.

In the same year Strauss<sup>17</sup> described atypical amyloidosis with particular involvement of the lymph nodes. Considerable amyloid was seen in the lungs in the large and small septa, and in the walls of the bronchi and blood vessels. There was considerable amyloid in the epicardium and endocardium but somewhat less in the myocardium. Symptoms of cardiac failure were not present.

Perla and Gross<sup>18</sup> in 1935 reviewed 112 cases of amyloidosis found in a series of 1500 autopsies. Three were considered as atypical, and in 1 of these, a patient with signs and symptoms of congestive heart disease, small amounts of amyloid were present in the lungs. The heart, tongue, kidneys and gastro-intestinal tract were more extensively involved.

The most recent example in the literature is the case reported by Reimann, Koucky and Eklund<sup>19</sup> in 1935. The patient had had low grade fever, progressive weakness, loss of weight, swelling of the tongue and evidences of cardiac failure for several months. Amyloid was prominent in the medium sized arteries of all the tissues examined. In the heart it was also present in the interstitial tissue with atrophy of many of the muscle fibers. Small plaques or beads of the substance were present in the alveolar walls of the lungs.

Primary amyloidosis of the heart without amyloidosis of the lungs has been described by Steinhaus,<sup>20</sup> Kann,<sup>21</sup> Beneke,<sup>22</sup> Warren,<sup>23</sup> Brocher,<sup>24</sup> Humphreys<sup>25</sup> and Budd.<sup>26</sup> It is noteworthy that symptoms of cardiac failure were noted in only 3 of these. Brocher's case was unusual because of the striking diminution of the caliber of the branches of the coronary arteries on account of the deposits of amyloid in the walls with resulting necrosis of the myocardium. Amyloid was not found between the muscle fibers of the heart.

## DISCUSSION

In Case 1, carcinoma of the bladder, chronic infection of the kidneys, and a localized bronchiectasis with suppuration may each have played a part in the etiology of the amyloidosis. This case, therefore, cannot be considered as an example of primary amyloidosis of the lungs. It is noteworthy that in two instances (Herxheimer,<sup>7</sup> and Hallermann<sup>19</sup>) localized deposits of amyloid were noted in the lung in association with an adjacent empyema. The amyloid in Case 1 was intimately associated with the area of suppurative bronchiecta-



sis which may have been directly responsible for its formation. The lack of involvement of other organs with the exception of the spleen was an unusual feature.

In Case 2, there was no evidence of any associated disease such as tuberculosis, syphilis, prolonged suppurative inflammation or extensive neoplasm. Perla and Gross<sup>18</sup> have suggested on the basis of the experimental work of others that a dietary peculiarity might play a rôle in the etiology of certain cases of atypical amyloidosis. As far as we have been able to ascertain from the history and post-mortem findings there was nothing unusual about this patient's diet. The amyloid deposits and the changes which they brought about were the only significant anatomical lesions. While amyloid was present in the spleen in considerable quantities the liver was little affected and the kidneys practically not at all. The organs unexpectedly involved were the heart and lungs. In the former the deposits were as extensive as those in any case we have found described in the literature, and undoubtedly in combination with the lesions in the lungs were the cause of the congestive heart failure. This clinical picture of myocardial failure with passive congestion of the organs and accumulation of fluid in the tissues and body cavities, produced by myocardial amyloidosis, has been observed by several writers and discussed, in particular, by Koller.<sup>15</sup> It is of interest that he noted also auricular thrombi, as did Larsen<sup>12</sup> and Perla and Gross.<sup>18</sup>

The presence of nodular deposits of amyloid in the lungs was a conspicuous feature in both cases described in this paper, although there were no masses of the size observed in the cases reported where amyloid was localized in the lungs. The unfixed heart and lungs in Case 2 reacted with a strong dark brown color to iodine, while a bronchial lymph node gave a much weaker reaction. Sections of paraffin embedded material from the lungs and several organs were stained with iodine, methyl violet, gentian violet, and Mallory's anilin blue stain. The amyloid was so faintly stained by iodine and by methyl violet that these methods had little value. In a frozen section of the lung stained with methyl violet the amyloid took a deeper lilac color. In paraffin sections the same reaction was seen with gentian violet, but the amyloid was not always well differentiated from other tissues. With the anilin blue stain the same difficulty was encountered. The amyloid, as well as the col-

lagen, stained a bright blue color. The combined Weigert's elastic and Van Gieson's connective tissue stain of the lung was the most useful in differentiating the various tissue elements in each case. The fact that alcohol was not used as a fixative may partly explain the unsatisfactory results with some of the methods used for demonstration of amyloid.

Let us now consider some of the features of pulmonary amyloidosis noted by other authors. As Kschischo,<sup>4</sup> among others, has pointed out, amyloid disease of the lungs may be divided into the localized form and that occurring in association with general amyloidosis. Examples of the former condition have been recorded by Herxheimer,<sup>7</sup> von Werdt,<sup>8</sup> Meyer,<sup>9</sup> and Hallermann,<sup>10</sup> while the other cases reported, including 2 presented here, belong in the second group, although in Case 1 the only other organ involved was the spleen. It is noteworthy that while the amyloid may be fairly widespread throughout the body, involvement of the liver, spleen and kidneys is seen in only about one-half of these cases. The heart is the site of amyloid in about the same number of instances, although the degree of involvement varies from slight deposits in the vessels to extensive interfibrillar masses.

From the standpoint of an obvious etiological factor we have again two groups. In the first of these there is no associated condition which would generally be considered as bearing a causal relationship. Most of the cases, including the 2nd one here reported, are examples. However, in the second group are the cases of Lubarsch and Plenge,<sup>6</sup> Herxheimer,<sup>7</sup> Schilder,<sup>2</sup> Kschischo,<sup>4</sup> Glaus,<sup>5</sup> Hallermann,<sup>10</sup> and Case 1, in which there is a presumably associated etiological disorder. Of these cases, 3 showed chronic pulmonary tuberculosis, 2 empyema, 1 multiple myeloma, 1 chronic inflammation of the lung, perhaps syphilitic, and 1 carcinoma of the bladder and localized bronchiectasis. It is noteworthy that in only 1 of these cases (Herxheimer's) was the amyloid limited to the respiratory tract. Here there was an associated empyema.

A study of the anatomical changes described by some of these authors reveals features of interest. Where the amyloid was grossly visible it was usually described as being homogeneous or hyaline, glistening or transparent gray or grayish yellow, and brittle. It was in the form of small nodules, streaks, or larger masses. In some instances an association with blood vessels or bronchi was observed.

The substance was distributed quite uniformly throughout both lungs or limited to isolated nodules or masses several centimeters in diameter in one or both lungs.

The lesions observed microscopically deserve consideration. The diffuse type, with which we are particularly concerned, varied from the scant deposits observed in the vessels and alveolar septa by Virchow,<sup>1</sup> to the extensive widespread amyloid degeneration of the lungs found in the 2nd case reported here. In almost all instances there was amyloid in the walls of the pulmonary vessels of small and medium size, involving especially the media. There was variation in the degree of participation of the capillaries of the alveolar walls, some authors having described amyloid occurring about them, while others claimed they were unaffected. Two or three writers mentioned an apparent involvement of the walls of lymphatics. Observations differed as to the participation of the alveolar walls. Some authors described the amyloid as being deposited in the connective tissue, while others, especially Schilder<sup>2</sup> and Kschischo,<sup>4</sup> found it beneath the lining epithelium in the form of fine streaks or rings. In other cases, described by Lubarsch,<sup>11</sup> Schilder,<sup>2</sup> Kschischo,<sup>4</sup> Glaus,<sup>5</sup> Larsen,<sup>12</sup> and Strauss,<sup>17</sup> the walls were so thickened by the amyloid deposits that the alveolar lumens were reduced in size, in some instances to mere slits. This change occurred in the cases here described. A cuboidal shape of the alveolar lining cells has occasionally been noted and in Kschischo's<sup>4</sup> case gave a glandular appearance to the lung. Involvement of the bronchi was observed by several authors, and in fact in Wild's<sup>13</sup> case the bronchi were affected while the alveolar walls were unchanged. The mucosa was usually involved and the amyloid was sometimes found around the mucous glands. Multinucleated giant cells were noted in close proximity to the deposits of amyloid by Herxheimer,<sup>7</sup> Meyer,<sup>9</sup> and Strauss.<sup>17</sup> The first two of these authors stated specifically that these giant cells did not contain any amyloid.

The two sexes were practically equally represented among the cases. The age distribution is of some interest. Aside from two individuals, 21 and 24 years old, both of whom had chronic pulmonary tuberculosis, all of the cases of amyloidosis of the lungs were in persons over 41 years of age.

There is no adequate explanation for the participation of the lungs in the changes of generalized amyloidosis in some cases, and their

lack of involvement in others. Most cases of advanced pulmonary amyloidosis may be considered as examples of atypical or primary amyloid disease, but in a number of instances amyloid has occurred in the lungs when there has been a characteristic generalized amyloidosis. Indeed, Lobel and Ten Thije<sup>27</sup> state that amyloid in the lung is not infrequently associated with amyloid in other organs. It seems highly probable that the minor grades of the condition are often overlooked or given but little attention. A small group of cases shows that advanced pulmonary amyloidosis without the usual precursors of this condition, especially when combined with extensive amyloidosis of the heart, may produce a fatal condition.

### SUMMARY

Amyloidosis of the lungs associated with general amyloidosis was first described by Virchow in 1857. Since that time a number of cases of pulmonary amyloidosis have been reported in some of which the deposits in the lungs have been part of a generalized amyloidosis, while in others the amyloid has been restricted to the lungs. In several instances these deposits were in the form of isolated nodular masses. Two new cases of amyloidosis of the lungs are reported here. In 1 of these, isolated deposits of amyloid were found in one lung, with more extensive amyloidosis of the spleen. A carcinoma of the bladder and localized bronchiolectasis of the affected lung were possible etiological factors. In the 2nd case there were unusually large quantities of amyloid in the lungs and heart and smaller amounts in many of the other organs. This case is an example of "atypical" primary amyloidosis since there was no recognized associated disease.

### CONCLUSIONS

1. Amyloid deposits in the lung are scant or absent in most cases of generalized amyloidosis, and occur about blood vessels or within the alveolar walls.
2. In some instances amyloid deposited in large amounts, frequently in nodular form, greatly alters the pulmonary architecture.
3. In most of such cases there is no preceding or concurrent chronic disease usually regarded as the precursor of amyloid.
4. Advanced amyloidosis of the lungs and heart may produce cardiac failure and death.

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## DESCRIPTION OF PLATES

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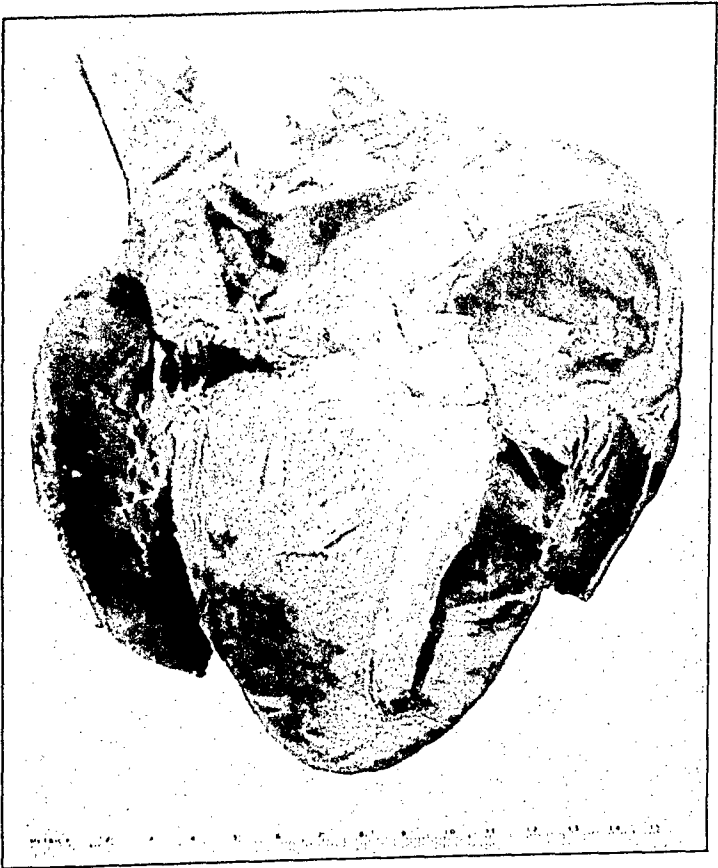
### PLATE 126

FIG. 1. Case 2. Gross photograph of the lungs, showing nodules of amyloid, especially in the lower lobes, with other large areas of homogeneous appearance.

FIG. 2. Case 2. The heart shows increased thickness of the walls and mural thrombi in the auricles.



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PLATE 127

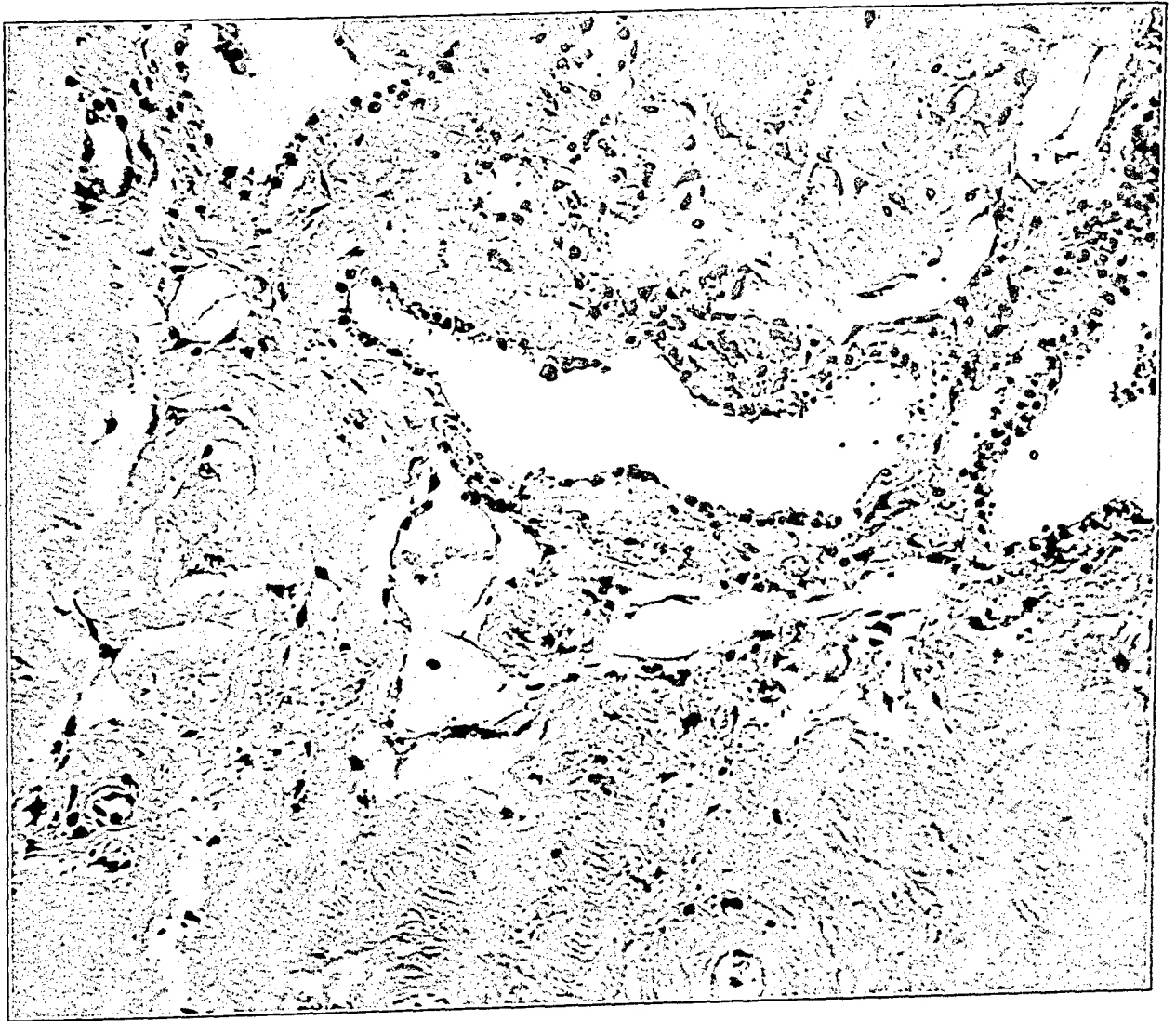
FIG. 3. Case 2. Photomicrograph of lung showing amyloid in alveolar walls and small arteries.  $\times 265$ .

FIG. 4. Case 2. Large masses of amyloid with partial or complete obliteration of alveoli. There is cuboidal metaplasia of the lining epithelium.  $\times 220$ .





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4



SCIENTIFIC PROCEEDINGS OF THE  
THIRTY-SIXTH ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS AND  
BACTERIOLOGISTS

HELD AT HARVARD MEDICAL SCHOOL,  
BOSTON, MASSACHUSETTS,  
APRIL 9 AND 10, 1936



# THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

## ABSTRACT OF BUSINESS SESSION

President WOLBACH in the Chair

The Secretary presented the nomination of the Council for officers as follows:

<i>President</i>	N. CHANDLER FOOT
<i>Vice-President</i>	E. B. MCKINLEY
<i>Treasurer</i>	F. B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	STANHOPE BAYNE-JONES
<i>Assistant Treasurer</i>	FREDERIC PARKER, JR.
<i>Assistant Secretary</i>	ALAN R. MORITZ

Voted unanimously to elect those nominated.

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Voted to elect the following new members:

Frank L. Apperly	Mendel Jacobi
Roger D. Baker	Abraham R. Kantrowitz
Howard A. Ball	Edwin H. Lawson
S. S. Blackman, Jr.	Max Lederer
Clark E. Brown	James R. Lisa
John W. Budd	Elizabeth Moore
Caspar G. Burn	David R. Morgan
Benjamin Castleman	Charles Oberling
John J. Clemmer	H. D. Palmer
G. Lyman Duff	Joseph G. Pasternack
Frederic H. Foucar	James H. Peers
Isadore E. Gerber	Joseph E. Pritchard
Harry S. N. Greene	Walter Putschar
Robert E. Gross	John R. Schenken
Bela Halpert	Arthur Schifrin
J. Beach Hazard	Martin Silberberg
Frederick A. Hemsath	Morris A. Simon
John D. Houghton	Joseph E. Smadel
Dudley A. Irwin	Lawrence Sophian

Paul E. Steiner

Ward W. Summerville

Joseph Tannenber

E. T. Thorsness

Phillips Thygeson

Arthur J. Vorwald

Ruth C. Wanstrom

Frederick W. Wigglesworth

Sigmund L. Wilens

It was also voted to reinstate Drs. C. L. Connor, F. E. Dolman, J. J. Moore, S. S. Schochet, and W. A. Starin.

Voted to accept with regret the resignations of Drs. R. L. Benson, A. E. Boycott, B. C. Crowell, F. T. Fulton, C. H. Hitchcock, A. P. Hitchens, A. Meyer, F. G. Novy, M. J. Rosenau, and W. C. Thro.

Voted to record with deep regret the deaths of Drs. G. C. Huber, R. V. Lamar, E. R. LeCount, and C. Norris.

Voted to nominate Dr. E. B. McKinley as representative of this Association in the Division of Medical Sciences, National Research Council.

Voted to adopt as the topic for the Symposium in 1937, The Genesis of Tumors. It was voted to appoint four referees and to request each one to present a paper limited to 20 minutes.

*Dr. C. V. Weller* is to be requested to talk on Heredity

*Dr. Leo Loeb* on Sex Hormones

*Dr. S. Burt Wolbach* on Immediate Response to Carcinogenic Chemicals

*Dr. Peyton Rous* on Filterable Agents

Voted to accept the invitation of Northwestern University Medical School to meet in Chicago in 1937, on Thursday and Friday, March 25 and 26, 1937.